Exploration of dynamic combinatorial chemistry in enzyme-inhibitor discovery

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Exploration of Dynamic Combinatorial Chemistry in Enzyme-Inhibitor Discovery

Submitted by Jelena Savović
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Signed.......Jelena Savović...................

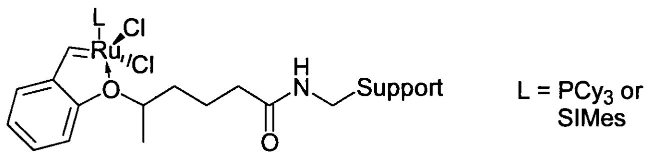
Date.......02/07/2003.........

Abstract

Dynamic combinatorial chemistry attempts to combine the statistical power of combinatorial chemistry with target-directed synthesis of drug candidates from building blocks *in situ*, thus bypassing the need for separate synthesis and screening of combinatorial libraries.

Concurrent synthesis and competitive binding of a three-component mixture of candidate Carbonic Anhydrase inhibitors was first attempted, based on literature precedent. A 19-fold amplification of the best to poorest binding component ratio is observed in the presence of the enzyme compared to the control experiment. Attempts to pursue this methodology further met with a number of associated problems which are briefly discussed.

Dynamic library generation requires employment of reversible reactions for connecting available building blocks under equilibrium conditions. Olefin metathesis is a reversible carbon-carbon bond forming reaction, which can be initiated by a number of organometallic initiators. Exploration of the use of cross-olefin metathesis for dynamic combinatorial library generation is attractive as alkene functionality is rarely found in common biological targets. This prompted work on the development of a suitable polymer-supported initiator for olefin metathesis that could be used for this purpose. Synthesis and testing of several such initiators is described and their advantages and disadvantages discussed.



The ligand was designed, synthesized and attached to different solid supports including some that can be used in polar solvents, such as TentaGel, Argopore and PEGA. These polymer-supported ligands were then loaded with the first or second generation ruthenium alkylidenes to afford polymer-supported initiators that could be used and recycled in a non-degassed solvent. Testing of the initiators with a range of substrates for ring-closing and cross-metathesis suggested how their design might be further developed toward their application in DCC.

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Publications

Dowden, J.; Savovic, J. Olefin metathesis in non-degassed solvent using a recyclable, polymer-supported alkylideneruthenium. *Chem. Commun.* **2001**, 37-38.

Abbreviations

δ	chemical shift (spectral)
ν_{max}	spectral number (IR spectroscopy)
Å	Ångstrom
A	absorbance
Ac	acetyl
Ar	aryl
ArH	aromatic proton
aq.	aqueous
Bn	benzyl
bp	boiling point
br	broad (spectral)
Bz	benzoyl
CA	carbonic anhydrase
Cbz	benzyloxycarbonyl
CM	cross-metathesis
Cy	cyclohexyl
ΔG	free energy
ΔG_a	free energy of activation
d	doublet (spectral)
DAB	1,4-diacetoxybut-2-ene
DCC	dynamic combinatorial chemistry
DCL	dynamic combinatorial library
DCM	dichloromethane
DEPT	distortionless enhancement by polarisation transfer
DIPEA	diisopropylethylamine
DMAP	*N,N*-dimethyl-4-aminopyridine
DMF	dimethylformamide

DMSO	dimethylsulfoxide
EDC	1-(dimethylaminopropyl)-3-ethyl carbodiimide
EI	electron impact
ESI	electrospray ionisation
Et	ethyl
FAB	fast atom bombardment
FTICR	Fourier-transform ion cyclotron resonance
GC	gas chromatography
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
IMes	1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene
*i*Pr	2-methyl-propyl (isopropyl)
IR	infra-red (spectroscopy)
J	coupling constant (proton NMR)
K_i	inhibition constant
LC	liquid chromatography
lit.	literature (reference)
m	multiplet (spectral)
M	moles per litre, molecular ion (mass spectrometry)
Man	D-mannose
m/z	mass to charge ratio (mass spectrometry)
Me	methyl
Mes	2,4,6-trimethylphenyl (mesityl)
mp	melting point
NA	neuraminidase
NANA	*N*-acetylneuraminic acid aldolase
NHC	N-heterocyclic carbene
NMR	nuclear magnetic resonance

3-NBA	3-nitrobenzyl alcohol (matrix in mass spectrometry)
OCT	olefin conversion technology
pH	logarithm of the concentration of H^+
Ph	phenyl
pNPA	4-nitrophenyl acetate
ppm	parts per million
q	quartet (spectral)
R_f	retention factor (in chromatography)
RCM	ring-closing metathesis
ROCM	ring-opening cross-metathesis
ROMP	ring-opening metathesis polymerisation
RP	reverse phase (chromatography)
r.t.	room temperature
R_t	retention time (in HPLC)
s	singlet
SIMes	1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene
t	triplet (spectral)
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultra-violet light
VIS	visible light
vs.	*versus*
WGA	wheat germ agglutinin

Contents

1 INTRODUCTION

1.1 Dynamic Combinatorial Chemistry - Introduction

1.1.1 Drug Discovery and Combinatorial Chemistry

Traditionally, drugs have been developed by stepwise synthesis of each individual compound, with subsequent testing for desired activity. However this process is extremely costly and time consuming and it is the increasing demand for the discovery of novel drugs that drives the development of innovative technologies towards this end. Combinatorial chemistry is an example of such a development.

Combinatorial chemistry[1,2] is a technique by which large numbers of structurally distinct molecules can be synthesised in a time and resource-efficient manner. The key feature of combinatorial chemistry is that compound synthesis is designed so that a range of analogues can be prepared using similar reaction conditions, either in the same vessel, or individually in parallel. In this way, many hundreds or thousands of compounds can be prepared in the time usually taken to prepare only a few by stepwise synthesis and modification. For example, if 10 acid chlorides are treated with 10 primary amines, a library of 100 amides can be generated. If this library of 100 amides is then allowed to react with 10 different alkylhalogens, under alkylating conditions, a library of 1000 tertiary amides is created. This demonstrates that the advantage of combinatorial chemistry lies in its statistical power to generate a large number of combinations from a few starting components. This process may be carried out using solution or solid phase chemistry, but for reasons of reaction yield and purity, the focus has mainly been on the use of solid-phase chemistry. In solid phase synthesis each substrate is linked to a solid support, such as polystyrene bead, thus producing physically separate products so that reagents and by-products can be simply removed by washing. The set of compounds synthesised by combinatorial chemistry is usually referred to as a library.

Combinatorial chemistry techniques allow testing of thousands of drug candidates using high throughput screening. Although this has revolutionalized drug development, the search for faster and more efficient testing methods continues. One promising method is *in situ* synthesis and screening of mixtures of drug candidates in *dynamic combinatorial libraries* (DCLs). The main innovation brought on by the concept of DCL is that the need to separately synthesise and test every member of the library is bypassed by the ability of a biological target to 'choose' from a pool of presented starting materials and drive the formation of only those combinations that express the highest affinity to the target. Any other combinations of starting materials are unlikely to be active.

1.1.2 Templated Synthesis and Dynamic Combinatorial Libraries

1.1.2.1 Thermodynamic versus kinetic control of reactions

A starting material (**A**) can undergo competing reactions resulting in different products (**B** and **C**, Figure 1-1).

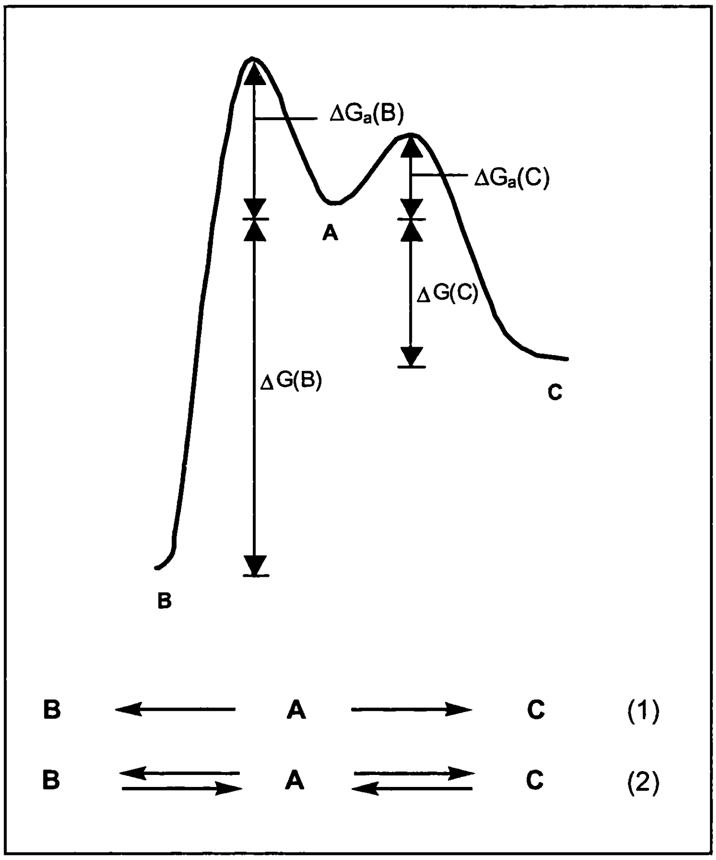
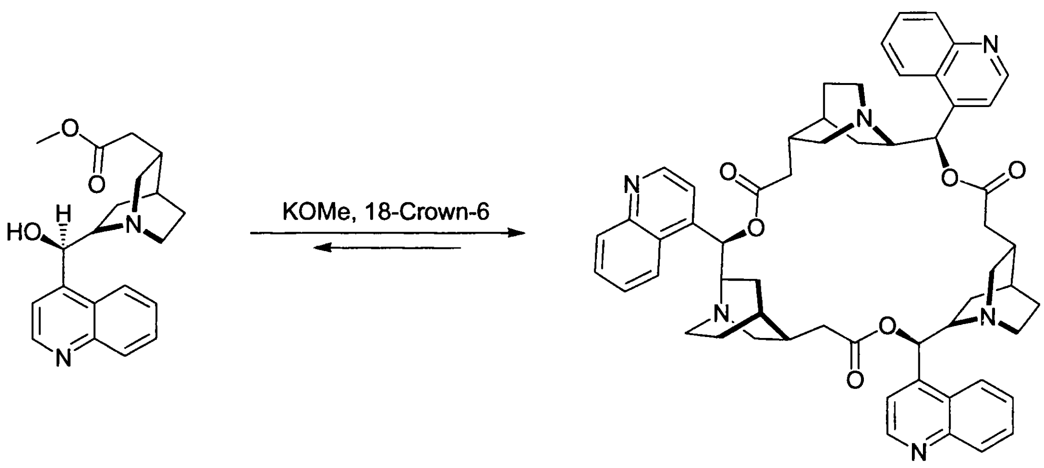


Figure 1-1: Free-energy profile showing kinetic *versus* thermodynamic control of product. Starting material (**A**) can react to give either **B** or **C**.

In this example product **B** is thermodynamically more stable than **C** (lower free energy ΔG), but **C** is formed faster (lower free energy of activation ΔG_a) as shown in Figure 1-1. If both reactions are irreversible (equation 1), **C** will be formed in larger quantities because it is formed faster. The reaction is then said to be *kinetically controlled.* However, if the reactions are reversible (equation 2) and allowed to reach equilibrium, the predominant product will be **B**. Under these conditions **C** is formed first but reverts to **A** much faster than thermodynamically stable **B**. The reaction is in this case *thermodynamically controlled.*

An example of thermodynamically controlled reaction is transesterification of the monomer **1**, derived from cinchonidine, in the presence of potassium methoxide (Scheme 1-1).[3,4] Although there are no kinetic barriers to formation of higher oligomers, the monomer is cyclized virtually quantitatively into the trimer **2**, as it is exceptionally thermodynamically stable. Control experiments confirmed that this reaction is reversible and under thermodynamic control.



1 **2**

Scheme 1-1: Transesterification of cinchonidine derivative **1**. Trimer **2** is thermodynamically favoured.[3]

1.1.2.2 Templates in synthesis

In synthesis, a template provides instructions for the formation of a single product from a substrate or substrates that otherwise have the potential to assemble and react in a variety of ways.[5] An example for templated synthesis is DNA replication, where one strand of DNA double helix acts as a template for the synthesis of the other strand.

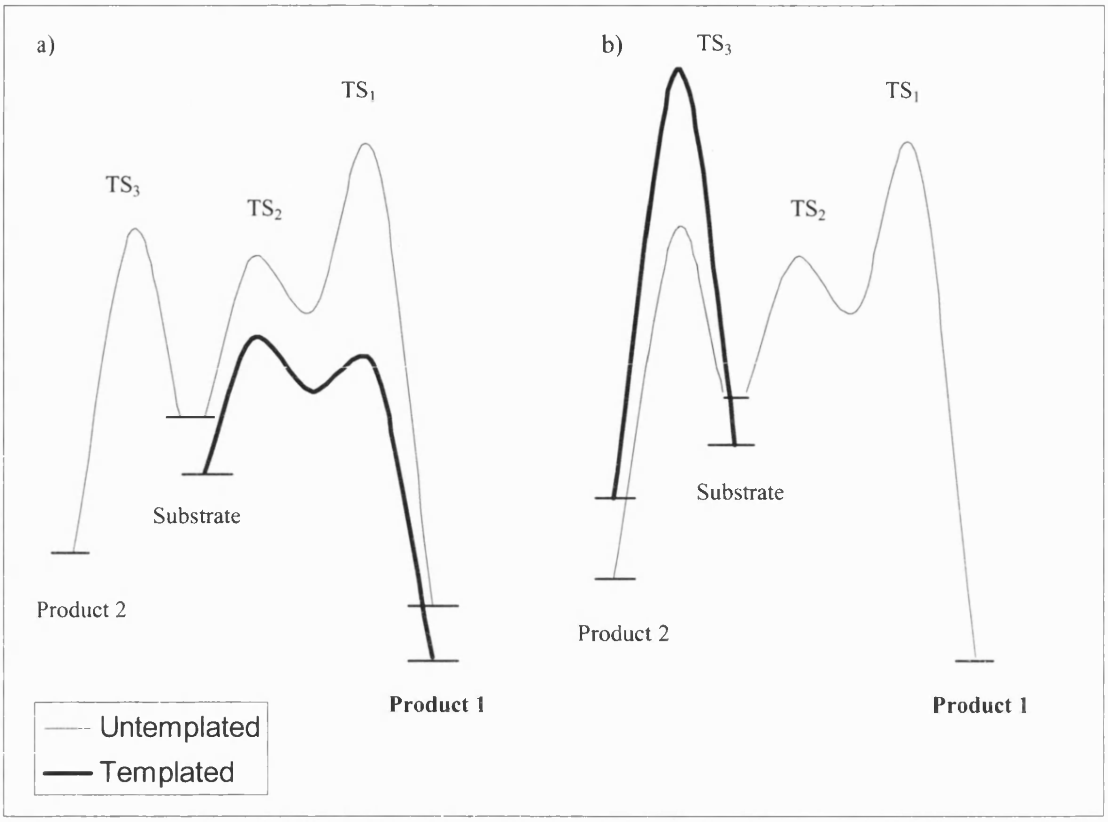


Figure 1-2: Energy profiles for untemplated and templated reactions under kinetic control. (a) Positive template reduces the energies of intermediates and the transition states (TS) leading to product 1. (b) Negative template increases the energy of TS_3 thus disfavouring the formation of product 2.[5]

A template that favours the reaction between substrates that are bound to it is a positive template. It is also possible for a template to disfavour the reaction between bound substrates, thus acting as a negative template. This negative effect is not caused by accelerating a competitive reaction, but by specifically disfavouring the formation of a particular product (Figure 1-2), as the substrates bind to the template in a way that does not allow the product to be formed. In other words, the template carries information on how the components should or should not interact.

1.1.2.3 Thermodynamic versus kinetic template

Templates can be either thermodynamic or kinetic depending on the reaction type.[5] Thermodynamic templating occurs in reversible reactions under thermodynamic control. The template added to a reaction mixture at equilibrium binds to one of the products and shifts the equilibrium toward further production of this species (Figure 1-3). If the reaction is under thermodynamic control, the only requirement for the template is that it should bind the desired product more strongly than all other species present in the equilibrium.

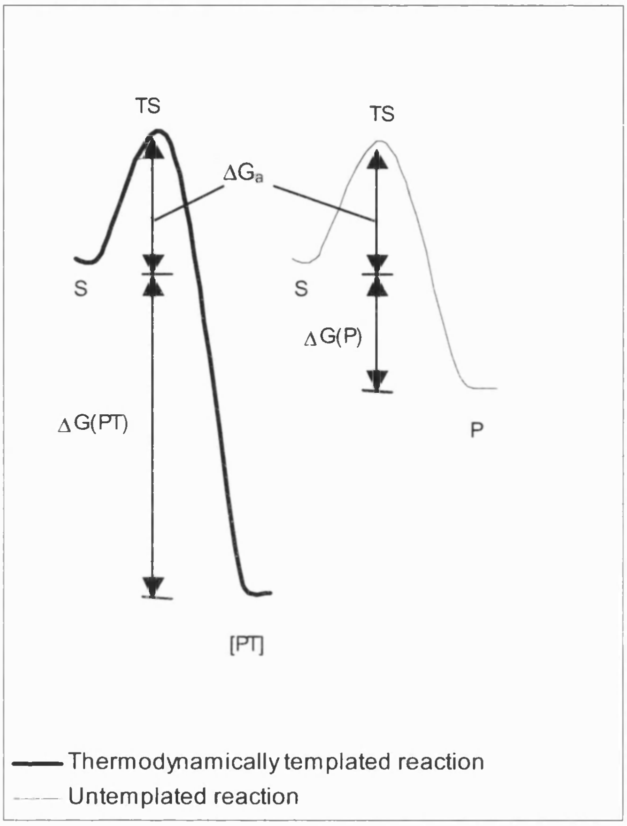
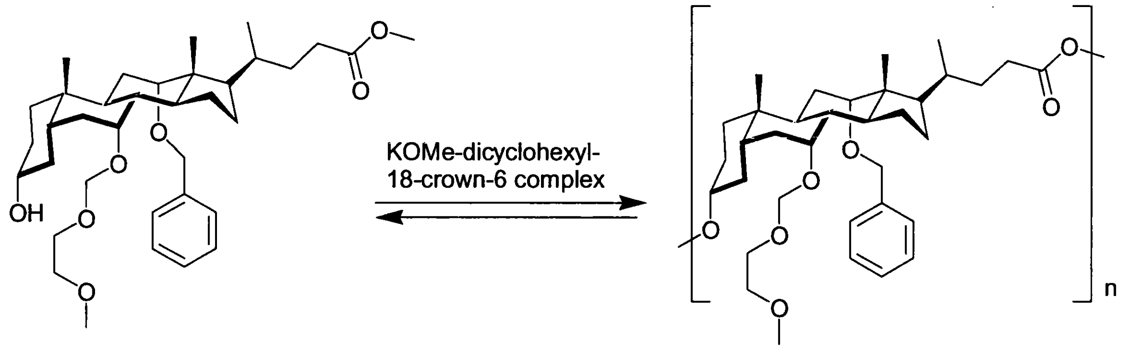


Figure 1-3: Templated *vs.* untemplated reversible reaction under thermodynamic control. Although free energy of activation (ΔG_a) is the same in both cases, the added template (T) binds product (P) to generate complex [PT] which is thermodynamically more stable than P (lower ΔG) and the equilibrium is then shifted towards further production of P (S - substrate, TS – transition state).

An example of a thermodynamic template is a metal ion templated macro-lactonisation of derivatised cholate esters.[6] Monomer **3** (Scheme 1-2) has a polyether side chain for recognition of a metal ion and a benzyl group for detection by UV. Reversibility of transesterification is dependant on the catalyst used and the best results were observed when potassium methoxide-dicyclohexyl-18-crown-6 complex was used with simultaneous removal of generated methanol as an azeotropic mixture. Equilibrium was achieved quickly and the proportion of trimer : tetramer : pentamer was 83:12:5 in the absence of a metal ion. When sodium iodide was added to the solution of monomer **3**, prior to addition of the catalyst, the distribution was shifted towards larger cyclooligocholates (61:24:15), as these give more thermodynamically stable sodium coordinate complexes.[6]



3
monomer

4
cyclic oligomer

Scheme 1-2: Metal ion templated macrolactonisation of the methyl cholate derivative **3**.[6]

Kinetic templates operate on irreversible reactions, so they have to stabilize all the transition states leading to the desired product (Figure 1-2). An ideal template of this kind would have both a positive effect on the desired reaction and a negative to all other reactions. Catalysts, including enzymes, are an example of a kinetic template as they act by reducing ΔG_a of a reaction thus making it faster. Enzymes bind to transition state more strongly than to substrate or product which allows for catalytic turnover. Most kinetic templates are not capable of this as they tend to bind the product more strongly.[5] If kinetic templates are used to control a product of a non-reversible reaction this is often referred to as *target accelerated synthesis.*

Templates can also be classified according to whether non-covalent or covalent interactions are formed between the template and the substrate. The former are usually referred to as *self-assembly* or *aggregation templated systems.*

1.1.3 Dynamic Combinatorial Libraries

Dynamic combinatorial chemistry (DCC) uses self-assembly processes to generate libraries of compounds - *dynamic combinatorial libraries* (DCLs). It relies on reversible processes for the generation of all possible combinations from a set of basic components, thus making available all structural features that these combinations may present. Since all the reactions are under thermodynamic control, the final product distribution will be influenced by the relative thermodynamic stability of each product and can be biased by the addition of different templates (receptors, targets).[7] The dynamic library however, has only the *potential* to produce all possible combinations of starting components, but not all of those combinations are necessarily present at any given time. The target 'drives' the creation of the preferred library member even if it may not be produced significantly in the absence of the target, hence one description of this process as a 'virtual' combinatorial library.[8]

In the presence of the target, the DCL component possessing the features best suited for binding to the target site will effectively be removed from the reaction. This sequestering causes a shift in chemical equilibrium towards further production of that ligand in order for the equilibrium to be maintained (Figure 1-4). Hence the product presenting the greatest complementarity with the template will be present in relative excess to the other components. Accordingly, this increase is detectable by quantitative methods such as HPLC, LC-MS and others. Amplification of the best species must be dramatic enough for definitive detection.

Dynamic combinatorial chemistry has also been described in literature as *molecular evolution*,[9] *thermodynamic templating*,[5] *receptor-assisted combinatorial synthesis*,[10] *target-guided ligand assembly*,[11] *target-accelerated combinatorial synthesis*,[12] *receptor-driven ligand evolution*, *targeted equilibrium shifting* and *adaptive chemistry*.[7]

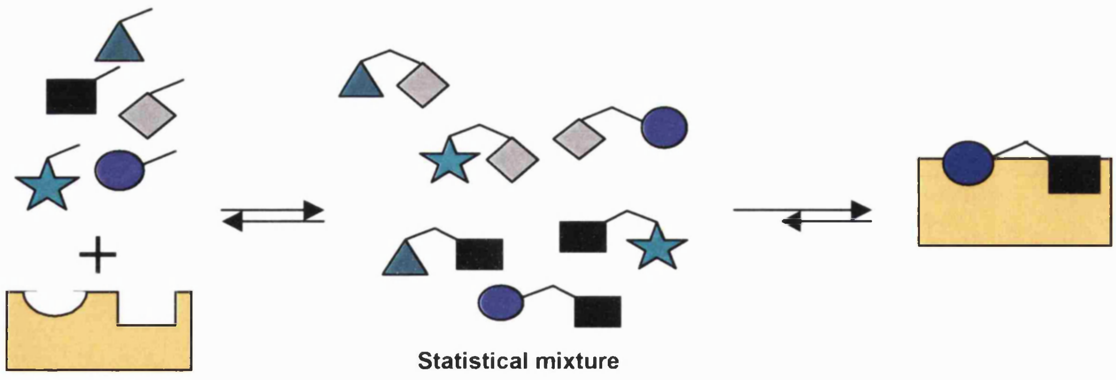


Figure 1-4: A cartoon representation of a dynamic combinatorial library in the presence of a template (e.g. a biological target).

1.1.3.1 Some examples of DCL applications

One of the first examples was reported by Eliseev and Nelen[13] in 1997. By photochemical interconversion of the three isomeric forms of an unsaturated dicarboxylate **5** (*cis,cis*; *cis,trans* and *trans,trans*) a three-component library of potential anionic receptors for arginine was generated. The equilibration and binding experiments were carried out separately using a two-compartment system (Figure 1-5).

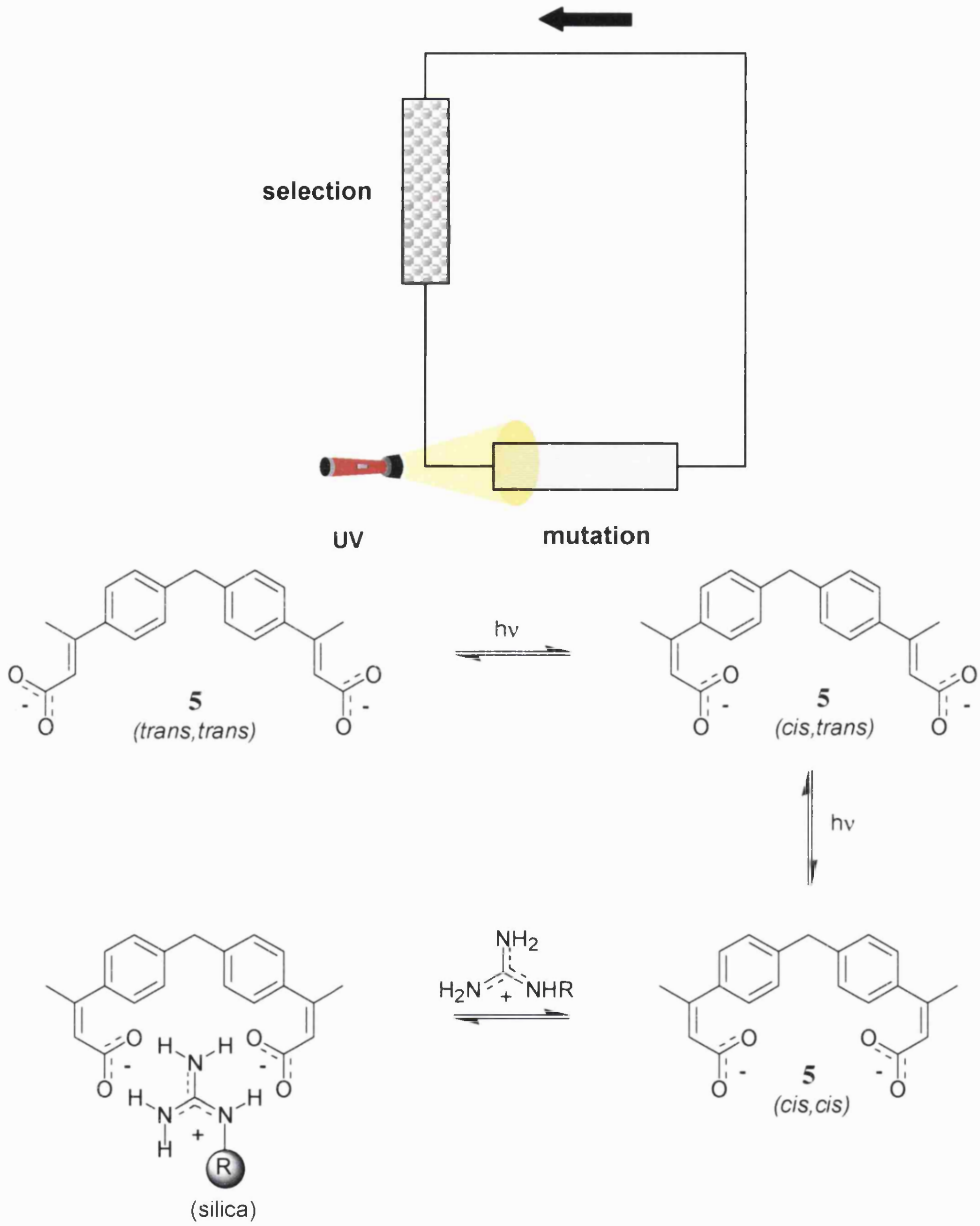
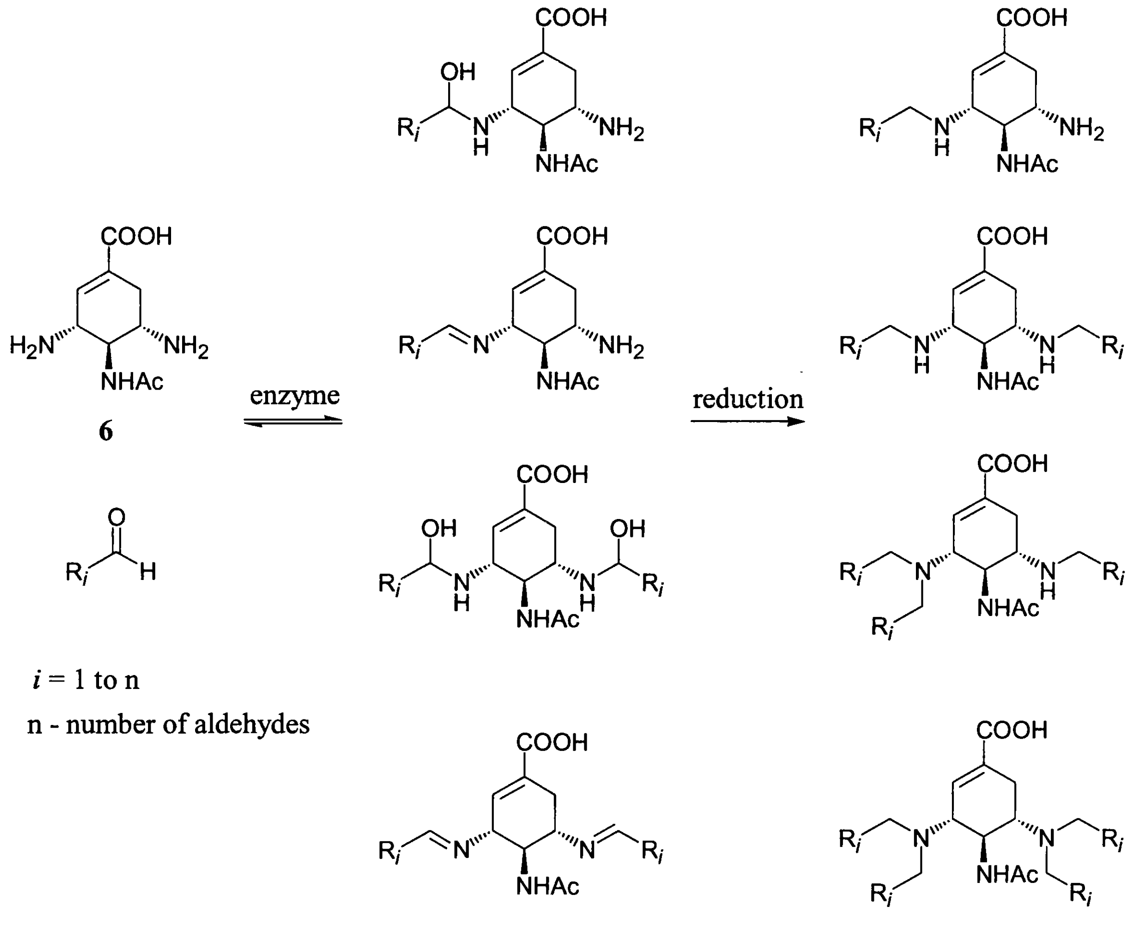


Figure 1-5: Schematic representations of the mutation and selection processes. Isomerisation of dicarboxylate **5** is induced by exposure to broad-band UV light followed by the selection *via* binding to silica-bound arginine (relative ratios of binding constants: $K_{cis,cis}$ / $K_{cis,trans}$ = 6.5; $K_{cis,cis}$ / $K_{trans,trans}$ = 100).[13]

Ultraviolet irradiation of the receptor resulted in a distribution of isomers (*cis,cis* : *cis,trans* : *tans,trans* = 3:8:89) which was then passed through an affinity column consisting of the arginine substrate bound to a silica gel support. The *cis,cis* isomer binds to the arginine and is retained on the column while the remaining mixture is exposed again to UV radiation in order to re-establish the equilibrium of the original distribution of isomers. After 30 successive isomerisation and selection cycles the bound receptor was washed from the column. The ratio of *cis,cis* : *cis,trans* : *trans,trans* was now 85:13:2. Although this experiment was not strictly a DCL, as the equilibration of the mixture (mutation) and binding to the template (selection) were not carried out simultaneously (*in situ*), multiple repetitions of the mutation-selection cycles compensate for this, thus making this process as close to being dynamic as possible. Physical separation of the equilibration (photo-isomerisation) and binding processes as two separate procedures, may be a convenient way for overcoming possible incompatibilities that could arise in DCC.

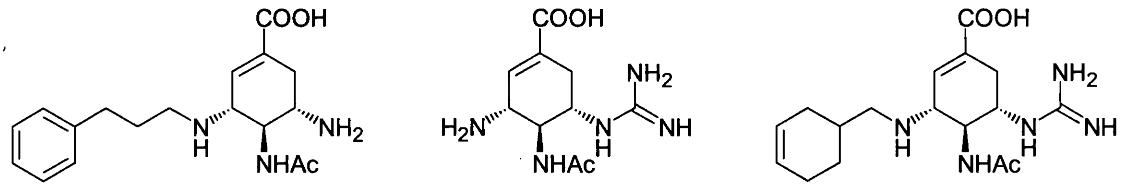
Ramstrom and Lehn[14] have used reversible disulfide interchange under mild aqueous conditions for creating two dynamic libraries of ten and twenty one disaccharides. These libraries were screened against the plant lectin concavalin A, which was either present *in situ* during library generation or added upon equilibration. Scrambling of the library in the presence of the target receptor resulted in higher yields of D-mannose homodimer and to a lesser extent D-mannose heterodimers, compared to the control. Similar shift in distribution, although with slightly lower yields, was observed when the receptor was added after equilibration.

Hochgürtel *et al.* have reported target induced formation of several potent neuraminidase inhibitors. Neuraminidase (NA) is implicated in influenza virus propagation and some of its potent inhibitors are already in use for the treatment of flu: zanamivir (Relenza™) and oseltamivir (Tamiflu™).[15,16] Their library is based on a common building block **6** (known to inhibit NA, $K_i = 31.3 \pm 4.5$ μM) and an array of hydrophobic aldehydes (Scheme 1-3). Reversible reaction between **6** and the aldehydes generated a library of transient imines and hemiaminals in the presence of target NA, which were subsequently irreversibly reduced with tetrabutylammonium cyanoborohydride and subjected to HPLC-MS analysis. Predominantly mono-substituted derivatives were detected in the presence of the enzyme, despite most mono, di-, tri- and tetra-substituted derivatives of **6** being detectable in the control experiment without NA. The most potent inhibitor from this library **7** (Figure 1-6, $K_i = 1.64 \pm 0.17$ μM), offered a 20-fold improvement in binding affinity compared to **6**.



Scheme 1-3: Target induced formation of neuraminidase inhibitors in a dynamic combinatorial library made of various hydrophobic aldehydes and 'scaffold' **6**.[17] Aldehydes react reversibly with amino groups of **6** forming transient imines and hemiaminals in the presence of neuraminidase; subsequent reduction with tetrabutylammonium cyanoborohydride fixes the library in preparation for HPLC-MS analysis.

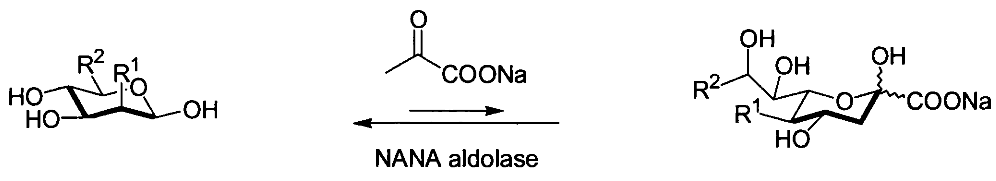
In the next set of experiments scaffold **6** was derivatised by replacement of the free amino functionality with guanidine group in order to improve affinity to NA. New scaffold **8** ($K_i = 352 \pm 32$ nM) was then equilibrated with a selection of aldehydes in the presence of NA, reduced and analysed in the same way. Indeed, the amplified species **9** proved to be a more potent inhibitor ($K_i = 16 \pm 2$ nM). Recently, similar work was published using **6** and an array of ketones instead of aldehydes in a quest of finding further NA inhibitors.[18]



7 **8** **9**

Figure 1-6: Inhibitors of neuraminidase.[17]

Recently, Lins *et al.*[19] reported the use of an enzyme-catalysed reaction to generate a dynamic library. In the presence of excess sodium pyruvate, *N*-acetylneuraminic acid aldolase (NANA aldolase, EC 4.1.3.3.) reversibly catalyses the conversion of 3 sugars (*N*-acetylmannosamine **10a**, D-mannose **10b** and D-lyxose **10c**) to generate a small equilibrated library of 3 products (sialic acid **11a**, ketodeoxynonulosonic acid **11b** and ketodeoxyoctulosonic acid **11c**, respectively, Scheme 1-4). Wheat germ agglutinin (WGA) was used as a template as it is known to specifically bind sialic acid (**11a**). When WGA was used *in situ* 80 % amplification of sialic acid is observed compared to the control without WGA. The authors point out that enzyme-catalysed reactions are reversible under physiological conditions and therefore ideal for use in DCLs as a tool for carbon-carbon bond formation. Furthermore, enzymes are easy to inactivate or remove from the equilibrated mixture, which allows a library to be analysed. An enzyme of broad specificity is needed in order to afford greater diversity of formed libraries.



10a R^1 = NHAc, R^2 = CH_2OH
10b R^1 = OH, R^2 = CH_2OH
10c R^1 = OH , R^2 = H

11a R^1 = NHAc, R^2 = CH_2OH
11b R^1 = OH, R^2 = CH_2OH
11c R^1 = OH , R^2 = H

Scheme 1-4: NANA aldolase catalyses the cleavage of sialic acid **11a** to ManNAc **10a** and sodium pyruvate; in the presence of excess sodium pyruvate reversible reaction takes place, so products **11a-c** are generated from the respective substrates **10a-c**.[19]

1.1.3.2 Reversible reactions, templates and building blocks in DCC

The essential step in dynamic combinatorial synthesis is the selection of an adequate reversible reaction.[8,20] Reversible interactions for DCC purposes can be classified in two major groups: covalent and non-covalent. At the molecular level components are exposed to a variety of covalent connections. Molecules containing carbonyl group (imines, esters, amides) are of great interest because they can undergo a number of reversible reactions under mild conditions. At least four types of carbonyl reactions may be utilised in DCC, including imine formation,[17,21] hemiketal formation, transacylation and aldol formation.

Imine formation was used by Huc and Lehn[21] in 1997 to generate a library of imines in the presence of enzyme carbonic anhydrase (CA) as a target. This experiment is described in more detail in chapter 2. The problem with imines is that they are generally unstable and therefore unsuitable for direct analysis. One solution to this is subsequent reduction of imines to amines, which can then be analysed. Also, amino functionality is abundant in biomolecules, so side reactions of aldehyde or ketone building blocks with free amino groups of target protein side chains are possible.

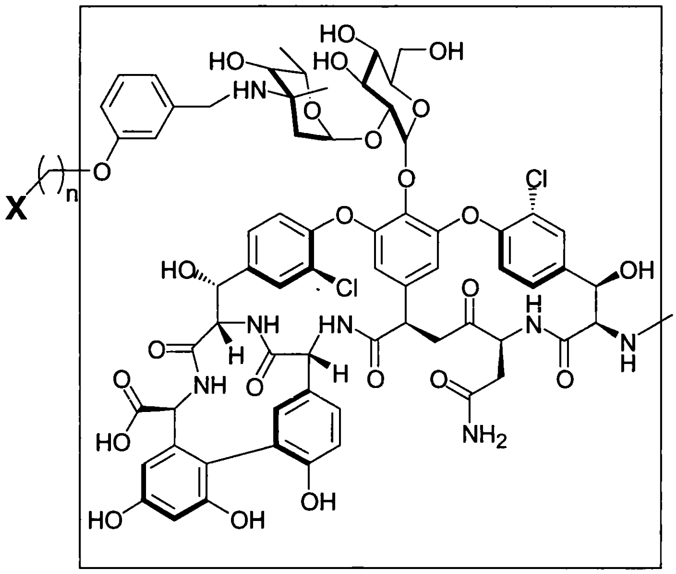
A reaction between hydrazines and a carbonyl is efficient and reversible, while *N*-acylhydrazones also offer hydrogen bond donors.[22] Dynamic libraries of oxime ethers have also been reported.[23-25]

Other possibly useful reactions to be used in dynamic library generation are disulfide exchange[14,26,27] or alcohol exchange in borate esters. As with the carbonyl reactions, disulfide exchange may be prone to side reactions with the target-protein as disulfide functionality is common in proteins. Michael and Diels-Alder reactions may also find use in DCC approach. Olefin metathesis may be used in the presence of a suitable initiator.[28]

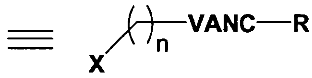
Out of all mentioned covalent reversible reactions only disulfide exchange and alkene metathesis are symmetrical. This makes them potentially more versatile as they are able to generate more diverse libraries. The reason for this is that the interactions between the components bearing the same functionality are allowed (including homodimerization). This does not occur in libraries assembled with, for example, two sets of building blocks with two different inter-reacting functional groups (e.g. under imine exchange conditions an aldehyde will react only with amines and not with other aldehydes). Olefin metathesis[10,29] has another potential advantage that may increase diversity and this is because scrambling creates a mixture of *cis* and *trans* isomers and therefore doubles the number of potential binding candidates, but also increases the complexity of analysis.

An example of alkene metathesis used in dynamic library formation was reported by Nicolaou *et al.*[12,26] Two dynamic libraries of vancomycin dimers, one using disulfide exchange of vancomycin thioacetate monomers and the other using monomeric vancomycin derivatives bearing terminal alkenes were generated under mild aqueous conditions (Scheme 1-5).

Vancomycin monomer derivatives



VANC



12 X = S-Ac
n = 2, 3, 4, 8
R = D-LeuNMe

13 X = $CH=CH_2$
n = 1, 2, 3, 7
R = H, β-Ala, L-Asn, D-LeuNMe, γ-Abu, L-Arg, L-Phe.

Vancomycin dimers

Ligation by disulfide bond formation:

12 **14**

n or m = 2, 3, 4, 8

Ligation by olefin metathesis:

13 **16**

n or m = 1, 2, 3, 7

Scheme 1-5: Dimerisation of vancomycin thioacetates (**12**) and vancomycin derivatives with terminal alkene functionality (**13**) to form disulfides (**14**) and olefinic dimers (**15**), respectively.[26]

Monomers with varied carbon chain lengths bearing the reactive functionality (thioacetate or alkene) and a different substitution pattern in the peptide chain (**R** in **12** and **13**, Scheme 1-5) were prepared as library building blocks. Both libraries were scrambled in the presence and absence (control) of peptide targets Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala to which vancomycin is known to bind. In the absence of the target peptides an expected statistical distribution of dimeric products is observed, whereas in the presence of target peptide Ac_2-L-Lys-D-Ala-D-Ala shorter tethered dimers with D-LeuNMe substitution are formed preferentially.

Reversible non-covalent interactions like metal coordination, electrostatic interactions, donor-acceptor interactions and hydrogen bonding could also be used for dynamic library generation. Intramolecular processes such as *cis-trans* isomerisation or conformational and structural interconversions (e.g. tautomerism) can also increase versatility of DCLs.[8]

Further increase in diversity of DCLs could be achieved by allowing the library building blocks to undergo multiple reversible processes either at the same time or in alternate manner. Goral *et al.*[30] have created 'double-level orthogonal' DCL in which two reversible interactions coexist independently of each other. The first level interaction was coordination of terpyridine-based ligands to the transition metal template and the second, imine formation with the aldehyde substituents on the terpyridine ligands. The two processes can be externally switched on and off by changing conditions such as pH, temperature or oxidation/reduction. Both reactions tolerate aqueous conditions and could potentially be used with a biological target *in situ*. Conversely, in 'non-orthogonal' libraries, multiple levels of interactions could take place at the same time (under the same conditions) and could interfere.

Various targets/templates have been used for different DCLs, including inorganic[31] and organic ions,[13,32] proteins (e.g. enzymes),[14,17,21,33] peptides,[12,26] and nucleic acids.[34,35]

Selection of suitable building blocks for the library formation is a key consideration and often depends on how much is known about the target. If information about the binding site of the target is available, the selection of a portion (if not all) of the building blocks would normally include structures that are known to bind to the target (recognition group). Obviously, building blocks also have to contain a reactive group. Finally, depending on the analytical equipment used to monitor the library generation, building blocks should also contain an analytically detectable marker (reporter group).[6]

1.1.3.3 Technical challenges of the methodology

An important consideration for designing a dynamic combinatorial library is that the selection of the starting components should be made to avoid reaction bias. Preferential production of certain library members must be due solely to receptor affinity and not due to differences in reactivity. This is a particular problem when the reactivity of building blocks varies substantially with substitution pattern of the reactive functionality, such as with alkene metathesis.[36]

This requirement for both reversible and unbiased reactions poses a limitation on the dynamic approach. The types of reactions available for library construction are thus restricted, as is the complexity of the library screen. If a biological compound is the target, its active form will only be permitted under certain pH, temperature and solvent constraints. A method of separating the biological target (e.g. enzyme) from the reaction, without compromising target-ligand binding could avoid this problem. Thus, introducing some form of compartmentalisation, such as the use of a semi-permeable membrane may allow more reactions and enzymes to be used. Some forms of compartmentalization have already been employed by some groups.[13,37]

Analysis and isolation of library members from vast DCLs can also present a problem. Techniques like HPLC-MS[17] or electrospray ionisation Fourier-transform ion cyclotron resonance tandem mass spectrometry (ESI-FTICR-MS/MS)[38] have proved to be of great advantage in analysing larger libraries. Isolation from a library is often not required if it is possible to identify the most abundant complex in the mixture. However, separate preparation of each possible individual library member may be required as a standard to aid its identification in the mixture. This can be extremely time consuming and is only possible for smaller libraries.

Identification of the 'fittest' species in the library is based on its relative amplification compared to the control experiment carried out without the target, which is proportionate to the differences in binding constants of the best binder and the other members of the library.[13] This may not be dramatic enough to allow selection of a single best binding species when library members have similar structures and thus similar binding constants.

Cheesman *et al.*[37] have developed a method for 'destruction of the unfit' that could further amplify the 'fittest' species and differentiate it from the other candidates. The authors synthesised and assembled a non-dynamic mixture of five dipeptides (four with sulfonamide functionality and one unfunctionalised control dipeptide). The screening experiment was carried out in a two-compartment vessel divided by a semi-permeable membrane. A solution of all five peptides in aqueous phosphate buffer was introduced to both compartments with the same starting concentrations, while bovine carbonic anhydrase (CA) was in only one of the compartments. Over time, the concentration of the peptide-sulfonamide with the highest affinity to CA increased in the CA containing compartment and decreased in the other. However, the concentration differences were too small to positively identify the best binder. Addition of a protease into the peptide compartment cleaved the weaker inhibitors, but the best inhibitor was protected by binding to CA in the other compartment and thus avoided proteolysis. As a consequence the strongest inhibitor was amplified. Although this was not a dynamic experiment, the methodology could be applicable to suitable DCLs. 'Destruction of the unfit' offers improvement in 'signal to noise ratio' of the information obtained form DCLs.

It is evident that there are ample difficulties associated with this methodology. That there are about as many reviews published in this area as there are experimental publications confirms growing interest in the area, as well as the complexity of experimental/technical issues surrounding it. A number of reviews are available for further reading in the area of dynamic combinatorial chemistry.[7,8,20,39-44]

Our interest in DCC was focused on the exploration of the concept itself and the use of suitable reversible reactions to generate DCLs in the presence of selected biomolecules. We were particularly interested to investigate the use of olefin metathesis as a tool for DCL formation and sought to adapt the existing olefin-metathesis initiators for this purpose. Olefin metathesis, its application and initiator considerations are discussed in the next section.

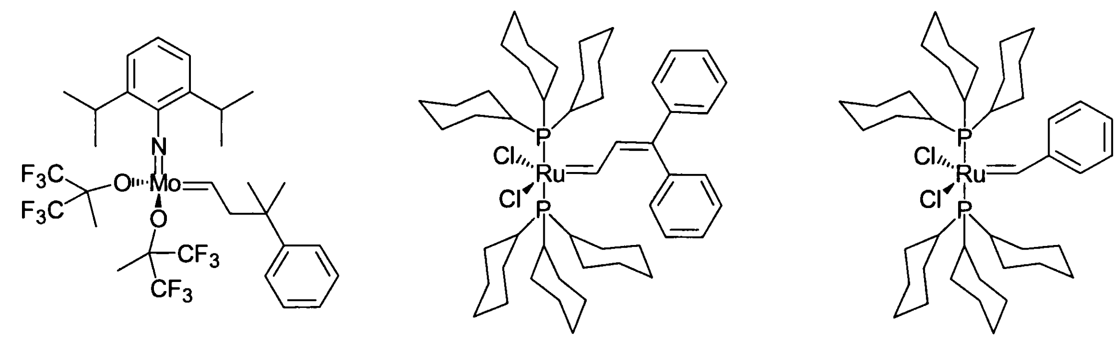
1.2 Olefin Metathesis - Introduction

Olefin metathesis is a carbon redistribution in which unsaturated carbon-carbon bonds are rearranged in the presence of metal carbene complexes and is a powerful tool for cleavage and formation of carbon - carbon bonds.[45]

1.2.1 History of Olefin Metathesis

Olefin metathesis was first observed in the 1950s by researchers in DuPont's petrochemicals department when propene passed over a molybdenum-on-aluminium catalyst afforded a propylene-ethylene copolymer and a mixture of propylene, ethylene and 1-butene as output gas. Over the 1950s and 1960s similar observations were reported by different groups from industry and academia using various poorly defined homogenous and heterogeneous 'catalysts' based on several transition metals (e.g. tungsten, molybdenum, rhodium, tantalum). The name 'olefin metathesis' was suggested by the Goodyear research team from Akron, Ohio, USA. The mechanism was a puzzle for a long time and several proposed mechanisms were later ruled out. The idea that the reaction may be initiated by a metal carbene was first proposed by Chauvin and Hérisson from the French Petroleum Institute in 1971. However this work went practically unnoticed until several groups reported experimental results that supported the metal carbene hypothesis in the period of 1972-1974. The most notable proof came form Casey and Burkhardt at the University of Wisconsin, Madison in 1974. They reported that the metal carbene (diphenylcarbene)-pentacarbonyl-tungsten reacts with isobutene to afford 1,1-diphenylethene as a major product.[36,46]

The first single-component homogenous catalysts for olefin metathesis were prepared during late 1970s and early 1980s. These were: the fore-mentioned (diphenylcarbene)-pentacarbonyl-tungsten made by the Katz group, bis(cyclopentadienyl) titanocyclobutanes, tris(aryloxide) tantalacyclobutanes and others.[36] The greatest contribution, however, to development of well defined metal carbenes for olefin metathesis was made by Schrock and Grubbs. In the late 1980s Schrock created several highly reactive 'metal alkylidenes' based on, first tantalum and tungsten and later molybdenum.[47] However, the most well known was molybdenum alkylidene **16** (Figure 1-7), known simply as Schrock catalyst.[48] This catalyst was highly active towards a broad range of substrates, but was highly air- and moisture-sensitive and had moderate to poor functional group tolerance.[45] In the 1990s Grubbs introduced first ruthenium based catalysts (**17** and later **18**) which are now widely used.



16 **17** **18**

Figure 1-7: First well defined molybdenum and ruthenium initiators for olefin metathesis.

1.2.2 Types of Olefin Metathesis and Its Applications

Depending on the type of transformation of the substrate(s) into product(s) induced by olefin metathesis three major types of reaction are observed: ring-opening, ring-closing and acyclic cross-metathesis (Figure 1-8).[45]

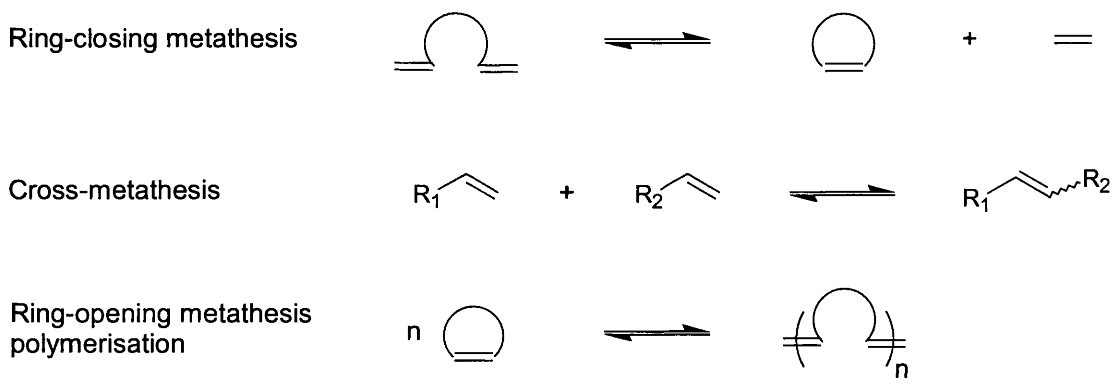
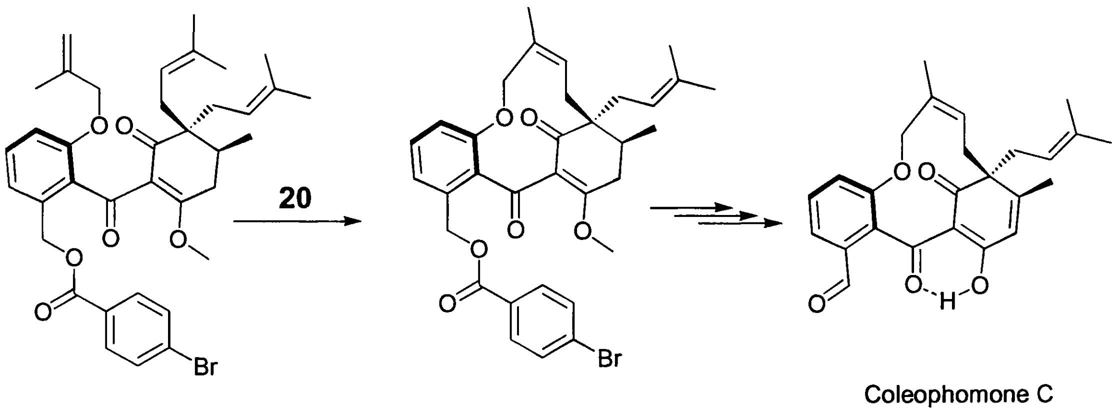


Figure 1-8: Applications of olefin metathesis reaction

Ring-closing metathesis (RCM) attracts a great interest for the synthesis of medium or large sized rings from acyclic dienes. The reaction is driven by the release of volatile ethylene, while ring strain influences product yield. As the reaction is reversible, the most thermodynamically stable product is formed. Macrocyclization metathesis of flexible large (>9) ring systems has provided a popular means toward the total synthesis of natural products such as the antifungal and antibiotic coleophomones B and C (Scheme 1-6).[49]



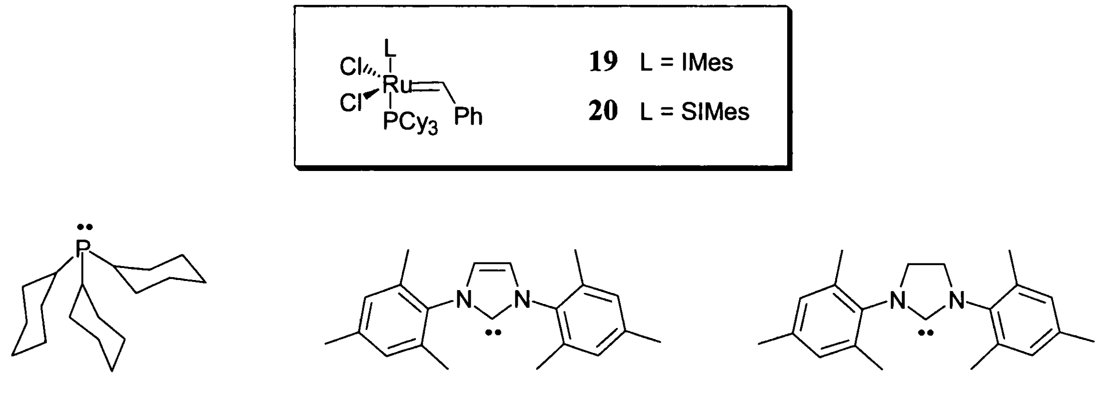
Scheme 1-6: Ring-closing metathesis of an 11-membered ring using second generation Grubbs' catalyst **20** (Figure 1-9) as a part of a total synthesis of coleophomone C – a natural product with antifungal and antibiotic properties.

Cross-metathesis (CM) between two different acyclic olefins potentially yields three new alkenes: a heterodimer alkene and two symmetrical products of self-metathesis, homodimers. In addition, this reaction generates a mixture of geometric isomers and methods for achieving stereoselectivity are of significant current interest.[45] The potential of cross-metathesis as a tool for the generation of libraries of alkenes was explored by Brandli *et al.*[29] Cross-metathesis of two internal disubstituted olefins generates a mixture of ten possible scrambled olefins (twenty including Z/E isomers). Cross-metathesis is industrially important as some of the essential alkenes, such as propene and 1-hexene are now made from other olefins using this reaction in a process called *olefin conversion technology* (OCT).[47]

1.2.3 Ruthenium-Alkylidene Initiators for Olefin Metathesis

In 1992, Grubbs introduced the first ruthenium based catalyst **17** (Figure 1-7).[50,51] The most important feature of this new type of catalyst is the improved tolerance to various functional groups. Ruthenium initiators react selectively with alkenes in the presence of acids, alcohols, aldehydes, ketones, esters and amides and are less moisture- and air-sensitive,[36] but they are also less active than Schrock catalyst. An improved version of this alkylidene ruthenium came in 1995 and is now widely known as Grubbs catalyst **18** (Figure 1-7).[52,53] Regardless of the somewhat lower activity level compared to molybdenum initiators, this 'first generation' of ruthenium initiators still has an enormous significance as it allows olefin metathesis to be carried out on a variety of functionalised substrates under mild conditions. They are also commercially available and relatively easy to use. This is demonstrated by a large number of publications that describe successful use of these initiators (especially **18**) in various synthetic applications.

In 1999 several groups reported a new generation of ruthenium based initiators in which one phosphine group was replaced with the imidazoline-2-ylidene ligand (IMes) **19** [54-56] and later with its saturated analogue (SIMes) **20** (Figure 1-9).[57] These catalysts retain functional group tolerance of their predecessors (**17** and **18**, Figure 1-7), but exhibit activity comparable to that observed in the earlier molybdenum based complexes (**16**, Figure 1-7), they are also more air-stable and have a longer half-life in solution. Unlike its predecessors (**17** and **18**), initiator **20** can induce ring-closing metathesis of tri- and tetra-substituted dienes, successfully initiate *ring-opening metathesis polymerisation* (ROMP) of low-strain and sterically hindered substrates and promote cross-metathesis to afford tri-substituted products.[36]



PCy3 IMes SIMes

Figure 1-9: Second generation ruthenium initiators bearing carbene ligands.

1.2.4 The Mechanism

The mechanism of olefin metathesis has been a subject of extensive study by Grubbs and several other groups.[58-63] Investigations of the influence of the ligands on activity and longevity of various ruthenium-initiators have contributed to elucidation of mechanism of ruthenium-catalysed olefin metathesis.

The mechanism proceeds in three phases: initiation, propagation and termination. Based on all experimental evidence so far, one of the recent studies[64] proposes a general mechanism of ruthenium-catalysed olefin metathesis reactions in which the binding of the olefinic substrate to ruthenium is preceded by dissociation of phosphine to form the

14-electron tetra-coordinate intermediate **A** (Figure 1-10) during the *initiation phase*. The tetra-coordinate species **A** is the active species that reacts with the olefin to start the catalytic cycle. However, at this stage the active species **A** can also re-associate to the free phosphine (deactivation) as well as coordinate the olefin, so these two processes are competitive. The co-ordination of the olefin to species **A** can proceed either *cis* or *trans* to the ligand L, but due to lack of experimental evidence Sanford *et al.* did not specify the geometry. The next step is the formation of the metallacyclobutane structure **B**. It is not yet clear if the metallacyclobutane (**B**) is a transition state or an intermediate.[64]

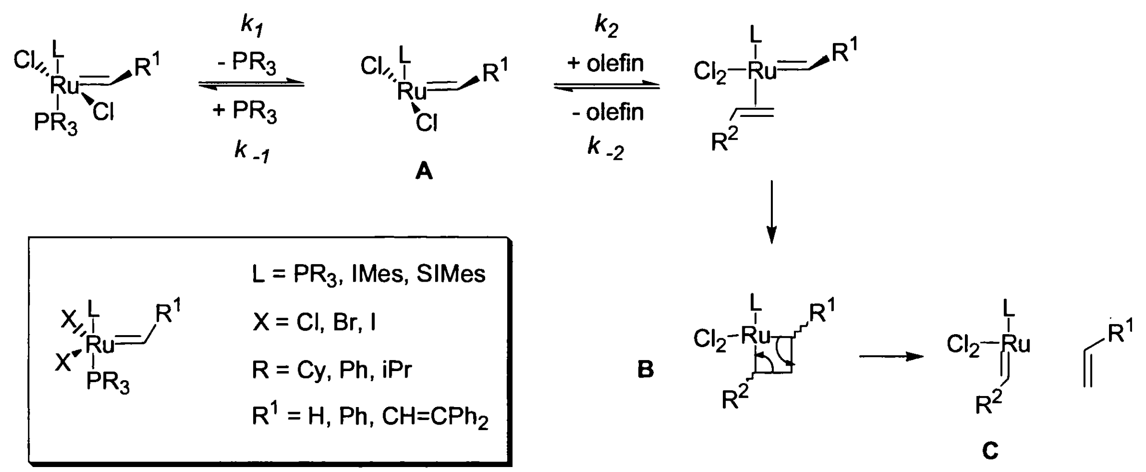


Figure 1-10: General mechanism of ruthenium catalysed olefin metathesis.

Cleavage of the metallacyclobutane affords a product alkene and the newly created propagating species **C** that still bears the remains of the alkene that was inserted into it. The propagating species then coordinates to a new substrate molecule and the reaction enters into the *propagation phase*. More than one propagating species can coexist during the reaction and these may have different stability and activity depending on the alkene substitution. Reassociation of the propagating species to the free phosphine is the *termination phase* (deactivation of the initiator) of the catalytic cycle. Thermal decomposition of the active species competes with the reassociation however, and the reaction eventually completely stops.

Several important observations resulted from these mechanistic studies. Factors contributing to overall activity of an initiator are the rate of initiation (which is related to the rate of dissociation of the phosphine) and the thermal stabilities of the complex and the propagating species. The activity of initiator $PR_3LX_2Ru{=}CH\text{-}R^1$ (Figure 1-10) depends on the identity of X and L type ligands. Changes in the steric and electronic character of the X- and L-type ligands influence olefin binding, phosphine dissociation and stability of the intermediates, causing differences in activity. Catalyst activity increases with the bulkier and more electron-donating phosphines and decreases with larger and more electron-donating halides.[36] Electron donating ability and steric bulk of phosphines facilitate dissociation of one of the phosphines and stabilisation of the 14-electron intermediate and therefore affects the initiation rate. The tricyclohexylphosphine (PCy_3) ligand (**18**) seems to achieve an ideal balance of basicity and steric bulk, as more basic and bulkier phosphines result in unstable complexes.[36]

As for the halides, changing the X type ligand from chloride to iodide leads to a 250-fold increase in initiation rates and this is predominantly due to the increase in steric bulk of X ligand.[64] Changing from chloride to bromide causes a less dramatic effect. However, a bulkier and more electron-donating halide also weakens the ruthenium-olefin bond and therefore disfavours olefin coordination, hence the overall effect of a larger and more electron-donating halide is loss of activity.[36]

The alkylidene moiety (R^1, Figure 1-10) affects the catalyst initiation rate as well as thermal stability of the catalyst. Alkyl substituted alkylidenes show better initiation than the methylidene complex, whereas the benzylidene derivative (**18**) is somewhere in between. The phenyl group is electron withdrawing, but its size favours dissociation of the phosphine.[36] Thermal decomposition of the methylidene complex follows first order kinetics and is independent of free phosphine concentrations. This is different from substituted alkylidene complexes which decompose through bimolecular pathways.[59]

This is of particular importance in RCM, as the propagating species in RCM is either an alkylidene $PCy_3Cl_2Ru{=}CHR$, where R is the attached substrate, or the methylidene $PCy_3Cl_2Ru{=}CH_2$, so its stability is important for propagation and longevity of the reaction. Initially, the catalyst reacts with an olefin of a diene substrate and then cyclizes to form the product and the methylidene, which then goes on to initiate cyclization of another diene substrate.[59] The half-life of methylidene species $PCy_3Cl_2Ru{=}CH_2$ in solution (C_6D_6, 55 °C) is 40 minutes, compared to the half-life of initiator **18**, which is 8 days.[59]

Addition of CuCl (or $CuCl_2$) increases the rate of metathesis initiated by ruthenium catalysts.[58] Copper is known to bind to free phosphines forming an ill-defined complex and this could promote the phosphine dissociation phase of the mechanism and therefore improve initiation. However, this also promotes thermolytic decomposition of the catalyst. The build-up of free phosphine slows down the initiation, but also slows down decomposition. The half-life of initiator **18** in the presence of insoluble CuCl (C_6D_6, 55 °C) is only 10 minutes.[59] In some cases rapid initiation compensates for the loss of stability.

Studies by the Grubbs group have revealed that the reason for the higher activity of the catalysts containing one N-heterocyclic carbene (NHC) compared to the bis-phosphine type catalysts is not, as previously suspected, because of the faster dissociation of the phosphine group (higher k_1, Figure 1-10). On the contrary, bis-phosphine type catalysts show much faster rates of phosphine dissociation than the second generation initiators.[60,64] However, after the rather slow dissociation of the phosphine, N-heterocyclic carbene ruthenium reacts fast with the olefin (higher k_2, Figure 1-10) and the higher turnover of olefins is achieved before the re-association with the free phosphine takes place (lower k_{-1}/k_2).[60]

Although the relative stability of the carbenes and metallacyclobutanes can change with reaction conditions, catalyst composition and alkene substitutions, the mechanism appears to be the same for all catalysts.[45] A recent paper by Vyboishchikov *et al.*[62] also confirms that for both types of catalysts (first and second generation) the dissociative mechanism with *trans* olefin coordination is favoured and that the rate-determining step is either the formation or cleavage of the metallacyclobutane **B** (Figure 1-10).

Adlhart and Chen[63] reported a combined quantum mechanical/molecular mechanics study of the olefin metathesis catalysed by the first and second generation ruthenium carbene complexes that were in agreement with the previous experimental findings of the Grubbs group. They found that the major topological differences between the potential surfaces for first- and second-generation catalysts are due to the different symmetries of the phosphine and NHC ligands. In the thermoneutral metathesis reaction with the first generation catalyst **18**, the rotation of the tricyclohexylphosphine (PCy_3) in all transition states is hindered by the unfavourable steric interaction with the large chlorine atoms. In the case of a second-generation catalyst (**20**), no rotation is needed because the NHC ligand has only twofold symmetry as opposed to the threefold symmetry of the phosphine ligand in the first generation catalyst. The authors conclude that the rate-determining transition state in the reactions catalysed by **18** (and other first generation catalysts) is the metallacyclobutane structure. For the reaction catalysed by the second-generation catalysts the rate-limiting step is phosphine dissociation.

1.2.5 Further Development of Olefin-Metathesis Catalysts

1.2.5.1 Ruthenium chelate complexes as initiators

Whilst exploring the mechanism of ruthenium-catalysed transformation of styrenyl ethers to chromenes, Harrity *et al.*[65] observed that ring-opening metathesis polymerisation (ROMP) of some substrates was not effectively initiated by **17** or **18** when 2-isopropoxy styrene (**23**) was present in solution (Scheme 1-7). Further investigation lead to discovery that the Ru-chelate complex **21** (Figure 1-11, Scheme 1-7) is formed *in situ*, which traps the catalytic species and reduces the rate of reaction. The complex was then synthesized separately by reacting 2-isopropoxystyrene with 1 equivalent **18** in dichloromethane (Scheme 1-7), isolated and characterised.

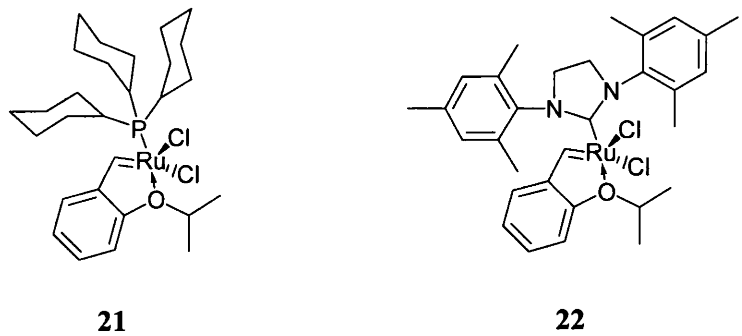
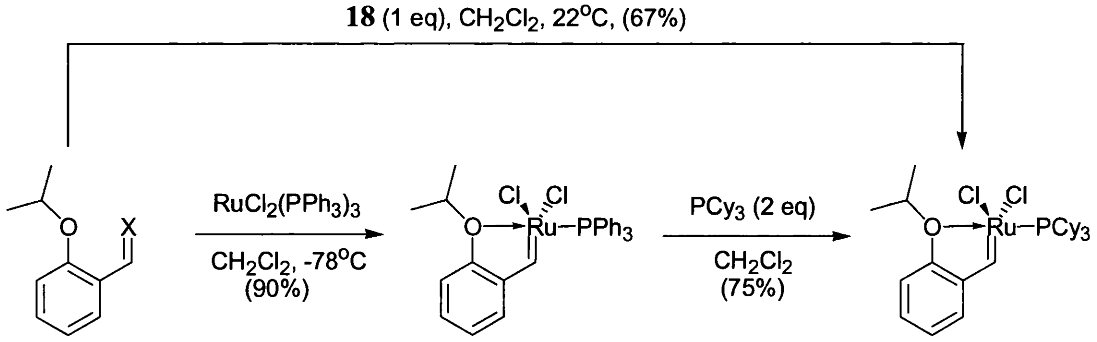


Figure 1-11: Hoveyda's ruthenium chelate initiators.

In a later publication from the same group,[66] further investigations revealed that this chelate is an active olefin-metathesis initiator that is remarkably robust and recyclable by column chromatography performed in an air atmosphere using reagent grade solvents. An efficient synthetic route that bypasses the need to use relatively expensive catalyst **18** as a starting material has also been described (Scheme 1-7). A two step synthesis from 2-isopropyloxyphenyldiazomethane (**24**) and $RuCl_2(PPh_3)_3$ affords **21** in a good yield.



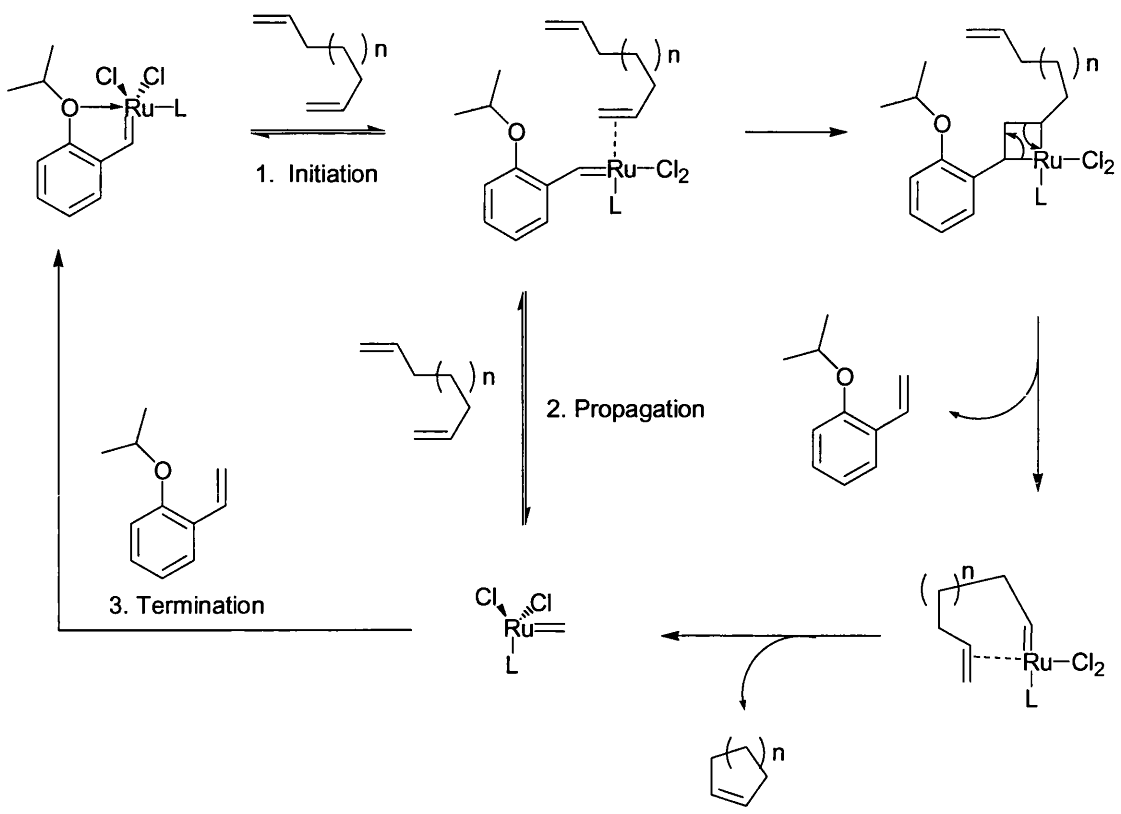
23 X = CH_2

24 X = N_2

21

Scheme 1-7: Syntheses of Ru-chelate catalyst **21**.

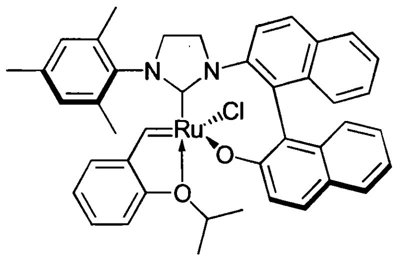
Replacing the isopropyl ether with a methyl ether gave a product that proved to be less stable and had poor catalytic activity. This indicates that the isopropyl group is important for the catalytic activity and robustness of complex **21**, most likely due to steric effects.[66] The initiator **21** maintained all the characteristics of its precursor (**18**) with the added robustness and recyclability. The authors have proposed a mechanism of RCM reactions catalysed by **21** (Scheme 1-8). The initiation phase starts by ruthenium–oxygen dissociation and substrate alkene coordination, followed by cleavage of 2-isopropoxystyrene, metallacyclobutane formation, cyclization of the product cyclic alkene and release of the active methylidene species that can either propagate by reacting with another diene or terminate by coordinating with 2-isopropoxystyrene (Scheme 1-8).



Scheme 1-8: Proposed mechanism of RCM initiated by Hoveyda style initiators

Following the discovery of 'second generation ruthenium alkylidenes' by Grubbs, Nolan and Herrmann's groups, in 2000 Hoveyda's group prepared several new Ru-chelate initiators bearing N-heterocyclic ligand (SIMes, Figure 1-9).[67] The most significant was initiator **22** (Figure 1-11), combining the robustness and recyclability of the styrenyl ether and the enhanced catalytic activity of N-heterocyclic ligand (SIMes). Blechert's group also reported synthesis and testing of initiator **22**.[68]

Recently, several groups have reported novel Ru-chelate complexes with further improvements in catalytic activity (Figure 1-12). Hoveyda's group[69] reported a recyclable chiral Ru catalyst for enantioselective olefin metathesis **25** (Figure 1-12). Chiral initiator **25** efficiently induces asymmetric ring-opening/cross-metathesis in air, in reagent-grade solvents and can be recycled.



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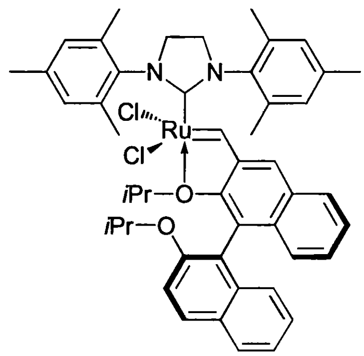

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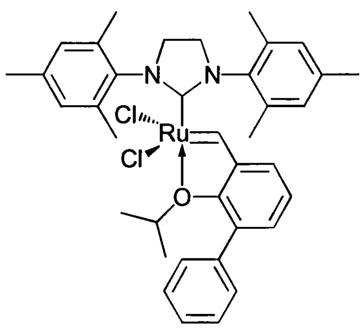

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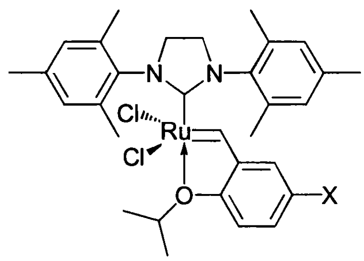


28 X = Br

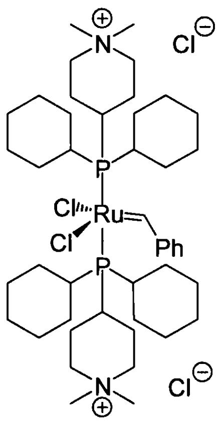
29 X = NO_2

Figure 1-12: Novel Ru-chelate complexes.

Wakamatsu and Blechert reported two complexes **26**[70] and **27**[71] (Figure 1-12) that are more active than both **20** and **22** (Figure 1-9 and Figure 1-11). Increased steric bulk of the BINOL- or biphenyl- based styrene improves dissociation of the styrene ligand, therefore improving initiation and allowing higher propagation turnover before re-association. A similar effect is observed by Grela *et al.*[72] upon introduction of a highly electron-withdrawing group (NO_2) in catalyst **29** (Figure 1-12), which is equally stable but significantly more active than **22**. They suggest that the decrease in electron density of the oxygen reduces its chelating capability and facilitates the formation of the active 14-electron intermediate, thus suppressing re-association. Bromo-derivative **28** was less active than **22**.[72]

1.2.5.2 Olefin metathesis in polar solvents

Olefin metathesis in polar solvents is an attractive goal and several attempts towards it have been reported. Although catalysts **17** and **18** (Figure 1-7) are tolerant to water, they are practically insoluble in it which makes it difficult to use them in aqueous conditions or in other polar solvents. However, there are a few reported experiments involving olefin metathesis with these catalysts in aqueous or two-phase organic-aqueous media.[12,26,73] In 1996, Grubbs group reported the synthesis of ionic, water-soluble ruthenium alkylidenes **30** and **31** (Figure 1-13).[73] These two complexes are soluble in water and methanol, but completely insoluble in benzene, tetrahydrofuran, acetone and ethanol. Complex **30** is also soluble in dichloromethane, but decomposes within several hours in this solvent. Both complexes are stable in degassed methanolic solution for weeks, but they decompose in aqueous solution in 2 days.[73] However, **30** and **31** are highly air-sensitive in solution and even traces of oxygen result in decomposition within minutes. As solids, they also decompose slowly in an air atmosphere, so they require storage and handling in an inert atmosphere.[74]



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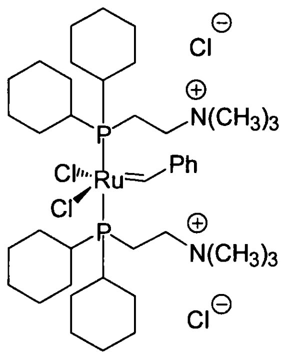

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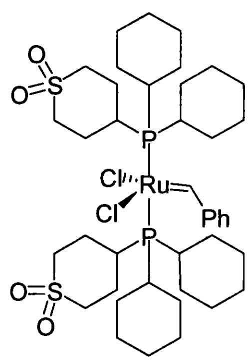


32

Figure 1-13: Water-soluble ruthenium alkylidenes.

Complexes **30** and **31** are reported to quickly and quantitatively initiate living ROMP of water-soluble monomers in water in the presence of Brønsted acid.[75] The authors stress that in the absence of the Brønsted acid the polymerisation is not 'living'. This is because the acid neutralises the hydroxide ions, which would otherwise cause catalyst decomposition, while protonation of the phosphine ligands enhances the activity.[75]

Ring-closing metathesis in methanol using ruthenium-alkylidenes **30** and **31** was also reported.[76] Both complexes react readily with acyclic olefins in protic solvents, but fail to cyclize α,ω-dienes due to instability of the propagating methylidene-ruthenium species generated in the first turnover. Successful RCM has however, been achieved using a substrate that generates a more stable propagating species after each turnover. Mono-phenyl substituted dienes (e.g. substituted diethyl diallylmalonate) have been found to give the best results as the more stable benzylidene-ruthenium active species is regenerated upon each turnover.[76]

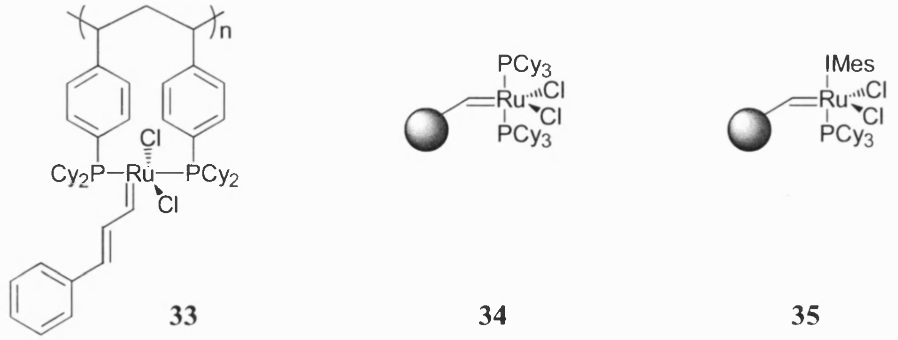
Most recently, Grubbs group have reported the synthesis of yet another homogenous ruthenium benzylidene complex **32** that initiates RCM in protic media (Figure 1-13).[77] As for previous polar initiators **30** and **31** complex **32** is also based on the benzylidene-ruthenium complex **18**, where the methylene unit in the position 4 of one of the cyclohexanes is replaced with a sulfone. This change was expected to have a major influence on solubility properties of the complex, but not to cause major disturbances in the electron-donating capabilities and bulk of the phosphine groups, which are known to play a key role in catalyst activity. The benefit of this initiator compared to the previous two is that it is not soluble just in water and methanol, but in almost any solvent due to its non-ionic nature. The complex is stable in methanol or benzene solution for several days at room temperature, but at 50°C it decomposes within 24 h. RCM of diethyl allyl(cinnamyl)malonate with 3 mol% of **32** was quantitative within 4 h in dichloromethane and benzene (at room temperature) and proceeded with a high yield (98 %) in methanol at 40°C after 12 h. However, a lower yield was observed (78 %) in aqueous methanol compared to neat methanol under the same conditions. Cyclization of unsubstituted diethyl diallyl malonate also proceeds quantitatively in dichloromethane at room temperature within 4 h.

Although these examples demonstrate that progress has been made in this direction, there is still a long way to go until wider application of olefin metathesis in protic (especially aqueous) media is accomplished. Additional advances in the field of olefin metathesis in protic solvents have been achieved by development of several polymer-supported initiators, including some of our work which is discussed in more detail later.

1.2.5.3 Polymer-supported and macromolecular initiators for olefin metathesis

Significant success has been made with the development of new highly active, stable and recyclable initiators for olefin metathesis, especially **20** and **22**. However, at the start of this project there were still a few areas waiting to be addressed including purification of the metathesis products and simplification of handling. Homogenous catalysts **18** and **20** are non-recyclable, are destroyed by reaction work-up and usually lead to highly coloured ruthenium residues in the product mixture which need to be removed by chromatography.[78] Immobilisation of an initiator onto a polymer support theoretically offers the ease of isolation of the relatively pure metathesis product by simple filtration. The recycled polymer bound initiator could be reused in the subsequent reaction. This would be of clear advantage in some areas, such as high throughput combinatorial chemistry. For these reasons several groups have sought to explore and develop polymer-supported initiators.

The first permanently immobilised ruthenium alkylidene **33** was reported by Grubbs[79] in 1995. This initiator was however, found to be at least two orders of magnitude less active than **18**, presumably due to the phosphine chelating effect.



33 **34** **35**

Figure 1-14: Grubbs' permanently immobilised and Barrett's 'boomerang' polymer-supported ruthenium initiators for olefin metathesis.

In 1999, Barrett's group made a polymer-supported 'boomerang' catalyst **34**,[78] by simply loading Grubbs' catalyst **18** onto a vinyl polystyrene suspended in dichloromethane for 1-2 h. This is a heterogeneous pro-catalyst that during ring-closing metathesis reaction releases homogenous catalytic species ($RuPCy_3Cl_2$) in solution and is subsequently recaptured by vinyl polystyrene when the substrate is consumed. The advantage of this is that compared to permanently immobilised **33**, better reaction rates are observed. Dried resin was indefinitely stable under normal atmospheric conditions.

Ring-closing metathesis activity of **34** was comparable to the parent homogenous catalyst **18**, yet isolation of the product consisted of simple filtration and evaporation. Ruthenium residues in the ring-closing product of diethyl diallylmalonate isolated in this way were just 500 ppm, compared to the unpurified product from a reaction initiated by Grubbs' catalyst **18** which were 5100 ppm (ICP-MS).[78]

Recycling of the resin was also attempted using diethyl diallyl malonate as a substrate, but the activity significantly dropped in the second run. The authors envisaged that this was due to the decomposition of the active methylidene propagating species and that the catalytic activity could be prolonged by addition of a terminal alkene into the reaction mixture. Indeed, addition of styrene or 1-hexene allowed for good conversion rates in two runs for most of the tested substrates and in some cases the catalyst was still active in the third run.

Barrett's group later reported the second generation 'boomerang' catalyst[80] **35** that showed improved rates of activity compared to **34** and retained catalytic activity for up to three consecutive runs without the addition of additive. Upon addition of additive (1-octene 10 mol% or 1-octene 10 mol% + PPh_3 5 mol%) the catalyst afforded good yields for various RCM products in three to four runs and was still active in the fifth run.

Development of polymer-supported initiators for olefin metathesis is a rapidly growing area and many further advances have since been made in this field. However, only developments that preceded and influenced the work described in this thesis were presented here. More recently reported polymer-supported initiators are briefly discussed later. Several reviews are available for further reading about polymer-supported catalysts in general and about polymer-supported initiators for olefin metathesis.[81-85]

1.3 Aims and Objectives of the Project

Exploration of dynamic combinatorial chemistry and investigation into its possible use for the discovery of enzyme inhibitors was the initial overall aim of this project. Initially, a literature based three-component competition experiment made through reversible imine formation in the presence of carbonic anhydrase (CA) was planned in order to familiarise with the methodology and gain experience in analysis and handling enzymes.

Exploration of alkene cross-metathesis as a tool for library formation was our next objective, which posed several challenges toward making this reaction available for use in conditions compatible with the presence of a biological target (enzyme). At the time, the available catalysts for olefin metathesis were reported to be relatively air-sensitive and could only be used in organic solvents, whereas the enzyme required buffered aqueous environment. Exploration of a two-compartment biphasic organic-aqueous system (using a Dynamic Dialyser) in which the enzyme (CA) would be present in one dialyser channel in aqueous environment, and the equilibrated library of alkenes in the presence of Grubbs' catalyst (**18**) in the other (organic) channel was planned.

Adaptation of the catalyst that would yield a robust, easy to handle, air-stable initiator that may be recycled was pursued next in order to attempt to improve the longevity of the cross-metathesis reaction under such challenging conditions. Attachment to different solid supports was to be explored to see if olefin metathesis could be carried out in protic solvents. Further, development of second-generation polymer-supported initiators was sought in order to achieve better rates of activity. Finally, application of these initiators to generate dynamic combinatorial libraries of carbonic anhydrase inhibitor candidates was planned.

2 DYNAMIC SELECTION OF ENZYME INHIBITORS - RESULTS AND DISCUSSION

Initially, our exploration of dynamic combinatorial chemistry started by repeating a literature based experiment involving a dynamic library of imines competing for the binding site of carbonic anhydrase II, present *in situ*.[21] The aim of this approach was to gain practice and familiarise with the methodology including analytical techniques, and to subsequently explore a two-compartment equivalent of this experiment and compare results. The second objective was to generate a new library of carbonic anhydrase inhibitor candidates, this time using olefin metathesis as a tool for bridging suitable alkene building blocks in the presence and absence of the enzyme.

Carbonic anhydrase (CA, EC 4.2.1.1) is a zinc metalloenzyme best known to catalyse the reversible hydration of carbon dioxide ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$), but it can also catalyse a variety of other reactions, for example hydrolysis of certain esters, including 4-nitrophenyl acetate (the basis of a standard method of analysis of its catalytic activity).[86] So far, fourteen different carbonic anhydrase isoforms have been identified in mammals and some of these are involved in physiological processes, such as respiration and transport of CO_2/bicarbonate between metabolising tissues and the lungs, pH homeostasis, electrolyte secretion, gluconeogenesis, ureagenesis, *etc.* Different isoforms are located in various parts of the cell; many in cytosol (CA I, CA II, CA III, CA VII), CA IV is membrane-bound, CA V in mytochondria and CA VI is found in saliva. Several isoforms without catalytic activity are also known.[87]

Inhibitors of carbonic anhydrase (mostly the CA II isoform) have been in use for many decades as therapeutic agents in the treatment of various diseases including glaucoma, epilepsy, mountain sickness and as diuretics. Recently, isoform CA IX has been associated with pathogenesis of certain tumours and CA XII, although present in many normal tissues is found to be significantly over-expressed in renal carcinoma. This explains recent increased interest in selective inhibition of certain CA isoforms.

Carbonic anhydrase CA II is the most studied isoform of the enzyme. At the base of its active site lies a zinc ion coordinated to His-94, His-96, His-119 and hydroxide ion in a tetrahedral coordination geometry. Zinc-bound hydroxide ion is hydrogen-bonded to the hydroxyl side chain of Thr-199, which is a H-bond donor to Glu-106. Transfer of the product proton to cytosol *via* shuttle group His-64 is required for catalytic turnover.[88] The substrate association site of CA II is a hydrophobic pocket adjacent to zinc-bound hydroxide, formed by residues of Val-143 at its base and Val-121, Trp-209 and Leu-198 at its neck.[89] Sulfonamides are potent CA inhibitors that coordinate to zinc at the catalytic site where the ionised sulfonamide nitrogen displaces the hydroxide ion bound to zinc, to form a stable enzyme-inhibitor complex. Interactions with the hydrophobic pocket are responsible for tuning selectivity. A general representation of a sulfonamide inhibitor interaction with the active site of CA II is shown in Figure 2-1.

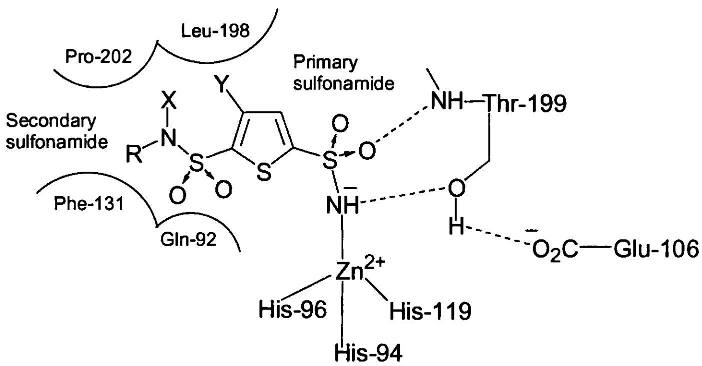


Figure 2-1: General scheme of sulfonamide inhibitor binding to CA II.[88]

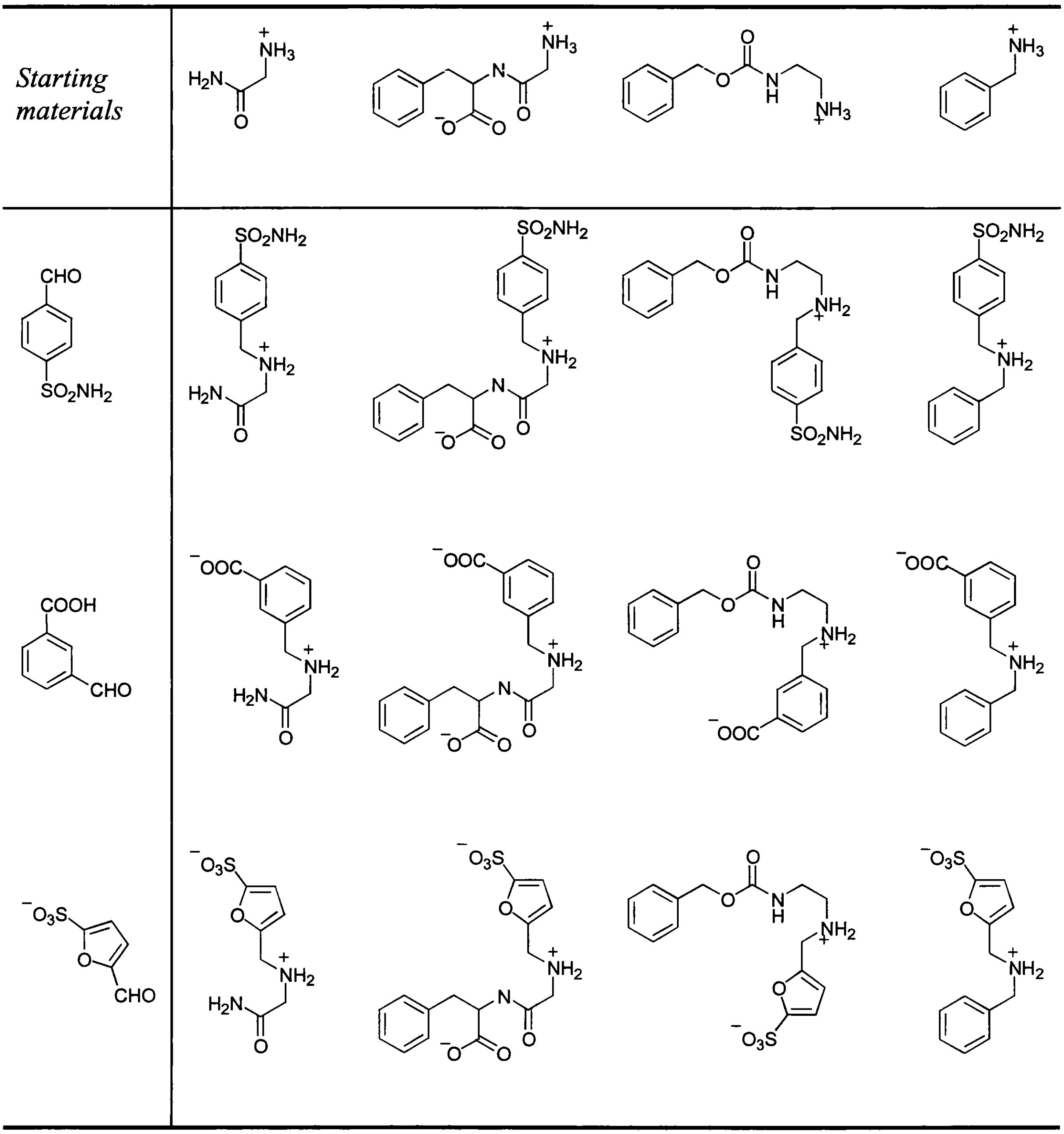
2.1 Imine Formation: Competition Experiment

2.1.1 Background

In 1997 Huc and Lehn[21] described a small dynamic library of imines (prepared from a selection of reversibly reacting aldehydes and amines) directed toward carbonic anhydrase. In concept, performing the reaction in the presence of the enzyme should select for the combination of reagents that bind most strongly to the enzyme. Monitoring changes in the distribution of products generated in the presence and absence of the protein should reveal the 'fittest' combination of fragments. In principle, this is a powerful method for discovering molecules with affinity to biological targets, such as enzymes and other protein receptors, and may be employed without extensive structural knowledge of the binding site. Though simple, this idea does bring a range of problems. These include the potential for bias in the reactivity of the components due to their structure; the problem of finding a sensitive analytical technique; the prospect of non-specific binding of the reaction components to other portions of the enzyme and the need to identify reaction conditions that are compatible with the protein target.

In their experiment, Huc and Lehn selected four amines (glycinamide, GlyPhe **36**, benzylamine **37** and *N*-Cbz-1,2-diaminoethane **38**) and three aldehydes (5-formylfuran-2-sulfonic acid, 3-formylbenzoic acid and 4-formylbenzenesulfonamide **39**, Table 2-1). These starting compounds were allowed to react with and without the presence of CA (1 equivalent) in 'near physiological' conditions (phosphate buffer pH 6, 20 mM, incubated at 25°C for 14 days). A ten-fold excess of amines compared to aldehyde and CA was used in order to consume all of the aldehyde and so prevent non-specific reactions with amino side chains on the protein. After reaching equilibrium, the enzyme was denatured and both experiments were analysed by reverse phase-HPLC. The products of the reaction are imines and are not stable enough to analyse by HPLC, so for practical reasons the imines were subject to reduction by addition of sodium cyanoborohydride. In theory, there are twelve possible reductive amination products, plus three primary alcohols (products of aldehyde reduction).

Table 2-1: A library of imines made from three aldehydes and four amines.[21]

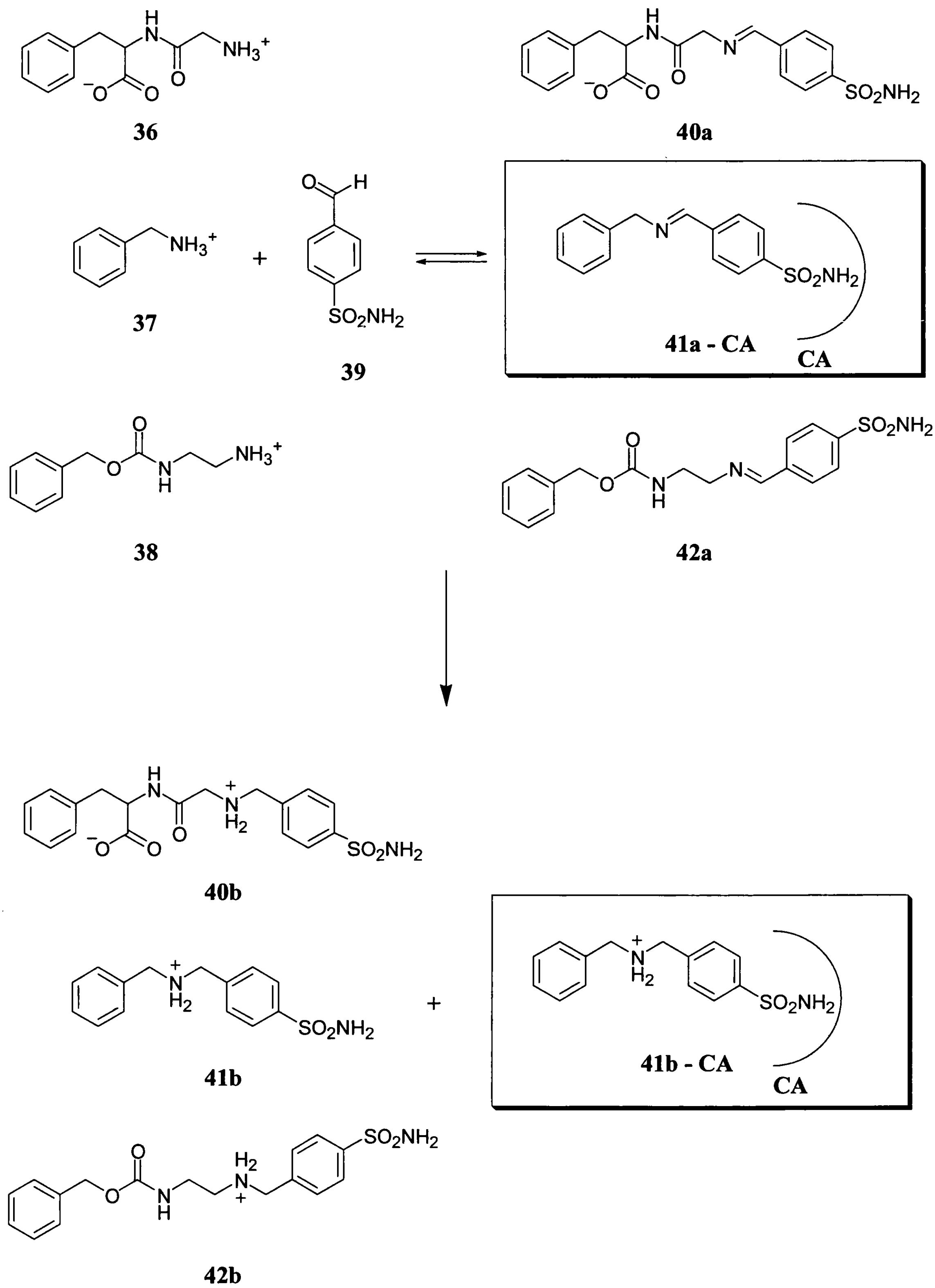


Being a zinc metallo-enzyme carbonic anhydrase is effectively inhibited by sulfonamide bearing molecules. Consequently, only products derived from the sulfonamide bearing aldehyde (**39**) have strong affinity for CA and this should be reflected by changes in their concentration when the reaction proceeds in the presence of the enzyme compared to the control. Products that bind to the enzyme are 'protected' from the reverse reaction and thus become amplified relative to other components of the library. Indeed this was observed by Huc and Lehn; only the products derived from the sulfonamide bearing aldehyde **39** were different between the control and enzyme reactions. The concentration of the GlyPhe derivative **40b** was unchanged, the benzylamine product **41b** was significantly increased, while the other two amine products were decreased. The sulfonamide bearing aldehyde is consumed by the benzyl amine to form the imine that binds most strongly to the enzyme binding site at the expense of the other amines. They later repeated the experiment with aldehyde **39** and only two amines (in five-fold excess) at the time in a direct 'competition' and compared them with the control. A twenty-one fold increase in **41b/42b** ratio in the experiment with the enzyme was observed, compared to control. Ratio of **41b/40b** increased five times in the experiment with the enzyme.

2.1.2 Initial Experiments

The initial aim of the project was to attempt to repeat the experiment described above, under identical conditions. This provided valuable experience of handling such experiments, allowed us to compare our results to those reported and provided a bench-mark for later experiments, such as those involving compartmentalisation.

Thus, a competition experiment was devised (Scheme 2-1), where one aldehyde (**39**) and three amines of similar reactivity and solubility (**36**, **37**, **38**) were allowed to react under similar conditions to form imines in the presence of the target biomolecule carbonic anhydrase (CA).



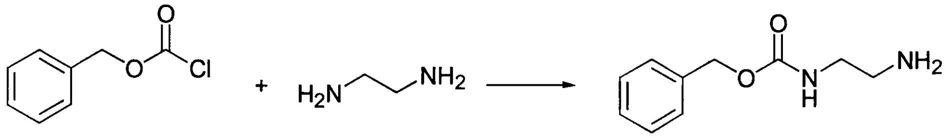
Scheme 2-1: The imine competition experiment. Reagents and conditions: bovine carbonic anhydrase (**CA**), sodium cyanoborohydride, phosphate buffer pH 6.

The starting aldehyde and product imines all contain sulfonamide functionality and thus 'compete' for binding to CA. Treatment with reducing agent under similar conditions to those reported yields amines that can be detected using reverse phase HPLC, so that the product with the highest affinity is amplified at the expense of the other amines. Again, the amines are used in five-fold excess to the aldehyde to overcome the abundance of side chain amino functionality of the enzyme that may provide potential spurious binding of the aldehyde.

2.1.2.1 Synthesis of starting reagents for imine library

Prior to conducting these experiments, each reduced imine product had to be constructed to provide standards for HPLC analysis, along with the starting amines.

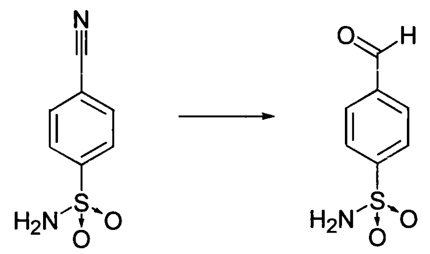
Monoprotected Cbz-ethylenediamine (**38**) was synthesised according to the literature (Scheme 2-2).[90] Ethylenediamine was treated with benzyl chloroformate at pH 3.8 under aqueous conditions, in the presence of methanesulfonic acid and sodium acetate. In a simple development, a pH-meter, rather than indicator was used in order to maintain the pH between three and four. In principle, under these conditions only one amino terminus is protonated per molecule, leaving the other free to react with benzyl chloroformate. Unfortunately, despite careful control of pH, a significant quantity of bis-substituted diamine was obtained. None the less, **38** was afforded as a slightly yellow oil that solidified on standing to provide an off-white powder in a disappointing 24% yield that decomposed after prolonged storage. The hydrochloride salt was used in later experiments to avoid this problem.



38

Scheme 2-2: Synthesis of **38**. Reagents and conditions: i. pH 3-4, r.t., 24%.

4-Sulfamoylbenzaldehyde **39** was made by reduction of 4-cyanobenzenesulfonamide using Raney nickel in refluxing formic acid (Scheme 2-3).[91] Compound **39** was obtained after precipitation from ethyl acetate in 51% yield as a pale yellow powder. Proton NMR showed a characteristic singlet for the aldehyde proton at 10.1 ppm.



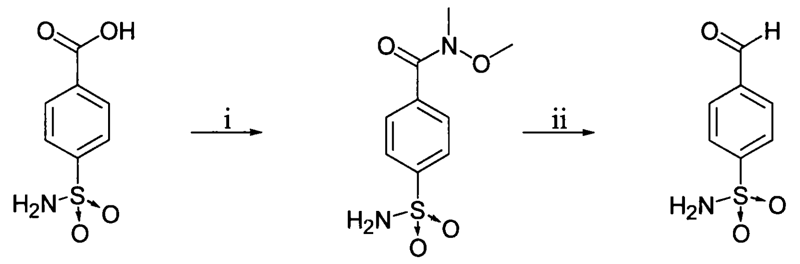
43 **39**

Scheme 2-3: Synthesis of **39**. Reagents and conditions: i. Raney Nickel, in refluxing formic acid, 51%.

The initial batch the aldehyde **39** was consumed and several attempts to re-synthesise it following the same procedure failed to afford pure compound. The reaction did not go to completion and separation of the product from the starting nitrile using column chromatography was unsuccessful (R_f(**39**)=0.5, R_f(**43**)=0.6, ethyl acetate : hexane 7:3). Repeated column chromatography caused degradation of the product. Hence, a new method was approached that bypassed the use of expensive 4-cyanobenzenesulfonamide and flammable Raney-Nickel. A two-step synthesis from 4-sulfamoylbenzoic acid proved to be cheaper, safer and reproducible.

N-Methoxy-*N*-methyl-4-sulfamoylbenzamide **45** was made from 4-sulfamoylbenzoic acid using *N,O*-dimethylhydroxylamine hydrochloride, 1-hydroxybenzotriazole, diisopropylethylamine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) in dimethylformamide affording Weinreb amide **45** in 51 % yield as a white powder (Scheme 2-4).[92] Compound **45** was reduced with lithium aluminium hydride in tetrahydrofuran, purified by flash chromatography and the residue precipitated by addition of hexane to its solution in ethyl acetate to give 4-formylbenzenesulfonamide

39 in 56 % yield. Reduction of the Weinreb amide to aldehyde is possible because, after the addition of one equivalent of hydride, the two oxygen atoms (from carbonyl and methoxy groups) coordinate to the metal ion, generating a stable five-membered cyclic chelate. Aqueous work-up hydrolyses the complex and the aldehyde is extracted into organic solvent.[93] This strategy has provided a reproducible route to aldehyde **39** from a cheap starting material, all be it in modest yields.



44 **45** **39**

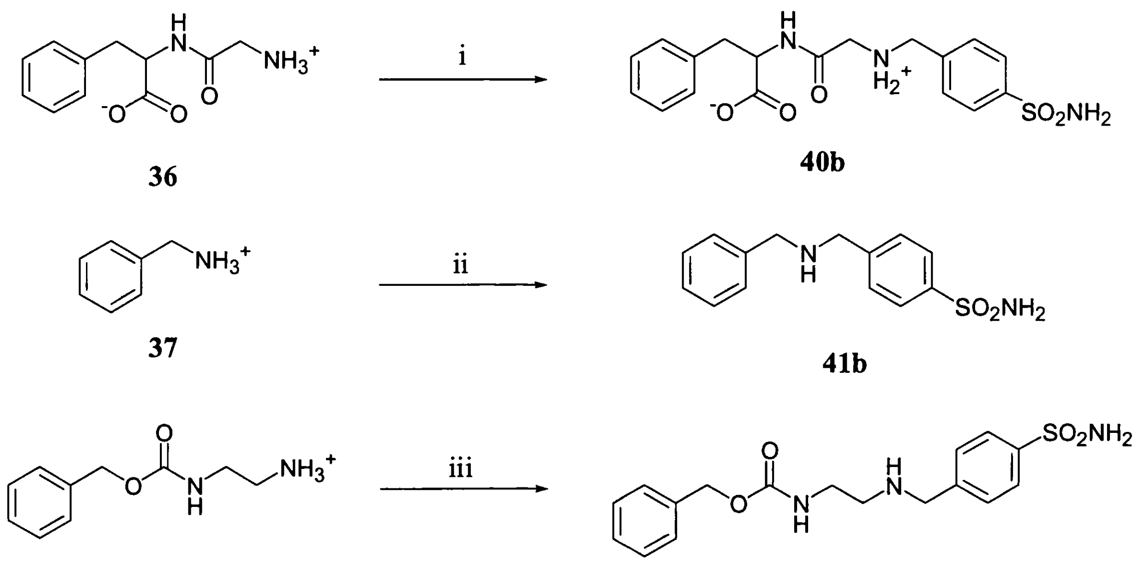
Scheme 2-4: Synthesis of aldehyde **39**. Reagents and conditions: i. *N*-methyl-*O*-methyl-hydroxylamine hydrochloride, 1-hydroxybenzotriazole, diisopropylethylamine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) in dimethylformamide, r.t., 51%; ii. lithium aluminium hydride in tetrahydrofuran, 0 to 25 °C, 56%.

2.1.2.2 Synthesis of HPLC standards for imine library

Standards of the secondary amines expected to form in the dynamic diversity experiment were needed as standards for HPLC analysis and were individually prepared (Scheme 2-5). Preparation of *N*-(4-sulfamoylbenzyl)-Gly-Phe **40b** was the most demanding of the three due to its propensity to form a zwitterion. Gly-Phe was treated with 1.2 equivalents of **39** in the presence of acetic acid and sodium triacetoxyborohydride[94] and the crude mixture then loaded onto a pre-filled Isolute C18 cartidge, eluted with water, eluent fractions combined and freeze-dried to afford **40b** in a 51 % yield.

For synthesis of 4-(benzylaminomethyl)benzenesulfonamide **41b**,[94] benzylamine was distilled under normal pressure and treated with **39** in tetrahydrofuran in the presence of sodium triacetoxyborohydride. Chromatography and recrystallisation afforded **41b** in 83 % yield as white crystals.

To obtain benzyl *N*-(2-(4-sulfamoylbenzylamino)ethyl)carbamate **42b**,[94] **38** and **39** were allowed to react in the presence of sodium triacetoxyborohydride in tetrahydrofuran at ambient temperature, purified by column chromatography and recrystallised from ethanol to afford **42b** in 37 % yield as white crystals.



Scheme 2-5: Synthesis of HPLC standards. Reagents and conditions: i. sodium triacetoxyborohydride, **39**, acetic acid, acetonitrile, r.t., 51 %; ii and iii. sodium triacetoxyborohydride, **39**, tetrahydrofuran, r.t.; ii. 83 %; iii. 37 %.

2.1.2.3 The experiment

Prior to each experiment the enzyme activity was tested spectrophotometrically using literature method by Pocker *et al.*[95] The concentration of the carbonic anhydrase stock solution was estimated spectrophotometrically using the extinction coefficient $\varepsilon_{280} = 5.7 \times 10^4\ M^{-1}cm^{-1}$.[21] Serial dilutions of stock solutions of starting materials (**36, 37, 38, 39**) and products (**40b, 41b, 42b**) were analysed by HPLC and a calibration curve was constructed for each compound. Three amines (**36, 37, 38**) were mixed with 4-formyl-benzenesulfonamide **39** and a reducing agent sodium cyanoborohydride in phosphate buffer. A five-fold excess of amines *versus* aldehyde was used to prevent side-reactions of the aldehyde **39** with the abundant amino groups on the protein. Both control and the experiment were incubated in a water bath at 25 °C for 14 days. During the incubation, samples from the control were occasionally taken for HPLC analysis. The control mixture reached equilibrium within a few days after which the product / starting material distribution remained constant. After 14 days the enzyme was denatured by heating to 80 °C and separated from the supernatant by centrifugation. Both experiment and control were then analysed by reverse phase HPLC, eluting by a binary gradient of acetonitrile and phosphate buffer.

Concentrations of starting aldehyde and the reductive amination products were estimated with the aid of calibration curves and the product concentration ratios from the reactions with and without CA compared. Unfortunately, due to the appearance of several new peaks in the chromatogram (Figure 2-2, possibly enzyme degradation products), it was not possible to resolve the GlyPhe alkylation product peak (**40b**) and subsequently determine its concentration in the reaction mixture. Thus, it was only possible to compare relative ratios of products **41b** and **42b** (Figure 2-2). The results obtained are summarized in the tables and complement the published results of Huc and Lehn (Tables 2-2, 2-3, and 2-4). We observed that the ratio of **41b** to **42b** was nineteen times higher in the presence of CA then in a control. Huc and Lehn observed this normalised relative ratio to be 21.[21]

Table 2-2: Concentrations of aldehyde **39** and reductive amination products after 14 days

Compound	*Control* (concentration, mM)	*With CA* (concentration, mM)
39	0.006	0.007
40b	0.34	-
41b	0.055	0.07
42b	0.06	0.004

Table 2-3: Concentration ratios of pairs of products

Concentration ratios	*Control* (ratio)	*With CA* (ratio)
40b/41b	6.2	-
42b/41b	1.1	0.06
41b/40b	0.16	-
41b/42b	0.92	17.5
40b/42b	5.7	-

Table 2-4: Normalised relative ratios [a]

Normalised proportions of the products	*Normalised value x*
$(\mathbf{41b/42})_{rel}$	19
$(\mathbf{41b/40b})_{rel}$	-
$(\mathbf{40b/42b})_{rel}$	-

[a] Normalized value $(\mathbf{41b/42b})_{rel}$ = 17.5 / 0.92 = 19 means that the ratio of two products is 19 times higher with CA then without CA (Lit.[21] $x = 21$).

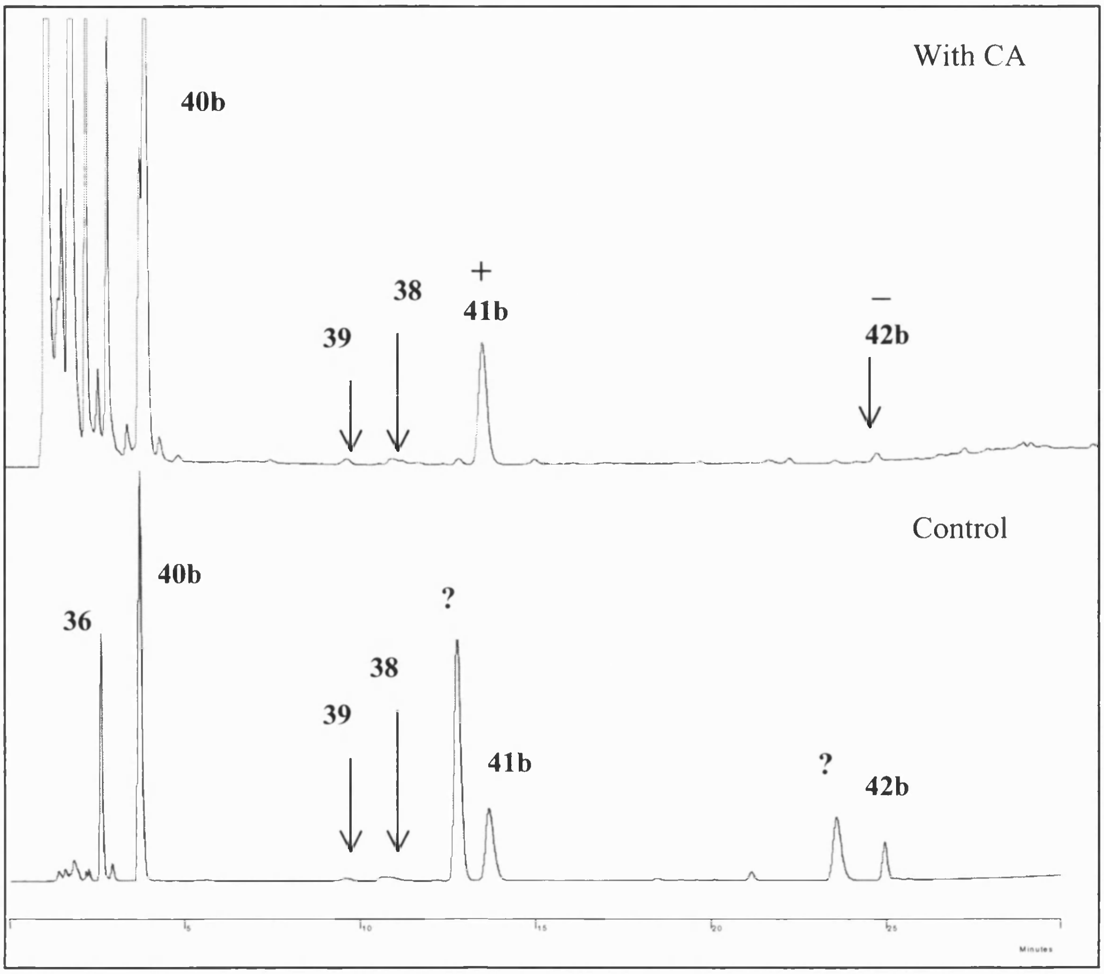


Figure 2-2: HPLC traces of the experiment with CA and control.

2.2 Compartmentalisation

One of the problems posed by the DCC approach is the requirement for the synthesis and screening to take place at the same time and in the same chemical environment. This means that reactions need to be carried out in a biocompatible environment that would mimic physiologically compatible conditions. This is not always possible as the reactions responsible for the interaction of the building blocks and the biological target may require different conditions (e.g. building blocks/reagents may not be soluble in aqueous environment, the enzyme may be inactivated/denatured in a solvent that is preferred for the selected reaction, *etc.*).

In theory, there are three solutions to this. Firstly, the enzyme (or another biological target) could be adapted to tolerate organic solvents, perhaps by immobilising the enzyme on a suitable support. Secondly, the reaction could be adapted to operate in the aqueous phase. Investigations into tailoring olefin-metathesis initiators to allow use in aqueous conditions could make this possible and this was one aspect of our project, which is discussed later.

Finally, a third possible solution involves introducing compartmentalisation to DCC, for example by physical separation of the equilibrating library from the enzyme by semi-permeable membrane (e.g. dialysis membrane).[37] Small molecules would transfer through the membrane and interact with the enzyme, whereas the enzyme, being a macromolecule would stay in its compartment. As a result the enzyme could be maintained in an appropriate buffered environment, and be 'protected' from the reaction conditions in the other compartment. This may allow library components and reaction to be present in organic solvent. In order to minimise the contact of enzyme with the reaction mixture, the reaction mixture would take place in a solvent that is immiscible with water, while the enzyme would be dissolved in buffer. This approach would be valuable in cases where the starting components and/or library members are insoluble in water/buffer. The partition coefficient of library members is a significant consideration in this process as differences between products may introduce bias into the library. The target biomolecule would have to be tolerant to small concentrations of the selected organic solvent, as the aqueous compartment would be saturated with the organic solvent depending on the physico-chemical properties of the solvent used. The same applies to the selected reaction; a moisture sensitive reaction would not proceed under such conditions.

Our initial plan was to apply compartmentalised approach to the fore-mentioned imine formation experiment involving imine formation in the presence of CA. The compartmentalisation could be achieved by using different pieces of equipment and one of these is a dynamic dialyser, normally used for purification of enzymes (Figure 2-3). If this was to be successful a two phase (organic/aqueous) olefin-metathesis scrambling experiment of a selection of alkene building blocks (including at least one bearing sulfonamide group) in the presence of CA would have been attempted.

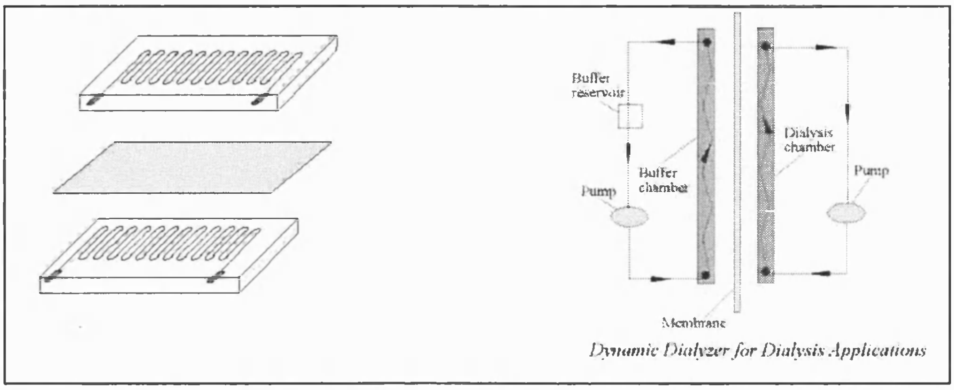
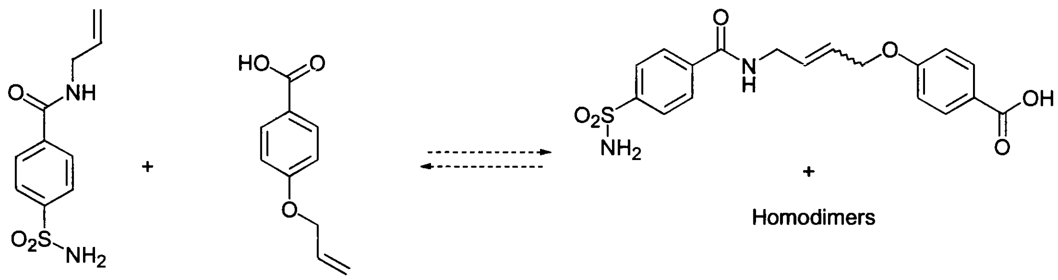


Figure 2-3: Dynamic dialyser (taken from www.amika.com with permission)

We have made several attempts to use a dynamic dialyser and one preliminary experiment is described. Two compounds bearing terminal alkenes (**46** and **47**) dissolved in phosphate buffer were pumped through one channel and CA through the other. The experiment was monitored by RP-HPLC and enzyme activity was monitored spectrophotometrically once per hour. The results were compared to control (without CA). We observed that a small amount of material is lost in control experiment due to either binding to the tubing or the membrane or undiscovered leakage. In the presence of CA disappearance of sulfonamide **46** is noted and decreased esterase activity of CA. There was no change in concentration of acid **47**.

The next planned step was to attempt cross-olefin metathesis of these two compounds in the presence and absence of CA (Scheme 2-6). This was not achieved as the olefin metathesis using catalyst **18** could not proceed in aqueous conditions so a two-phase organic/aqueous system in a dynamic dialyser was contemplated. However, even the reaction in organic solvent (without enzyme) was a problem due to poor solubility of sulfonamide **46** in dichloromethane and toluene, the usual solvents for olefin metathesis, so this part of the experiment was not developed. At this stage we started considering the options of developing an initiator that could effect olefin metathesis in aqueous environment.



46 47

Scheme 2-6: Attempted cross-metathesis of sulfonamide **46** and acid **47**. Reagents and conditions: Grubbs' catalyst **18**, r.t.

Technical problems with the use of dynamic dialyser were also encountered, associated with the uneven pumping of the two channels, which ultimately forced the contents of one channel into the other. This meant that the experiment had to be done within a few hours and under continuous supervision and adjustment of the pumping speeds in both channels. As the imine formation experiment required several days to equilibrate this would have not been possible. We considered some other options to achieve compartmentalisation, but this part of the project was unfortunately left unexplored in the end.

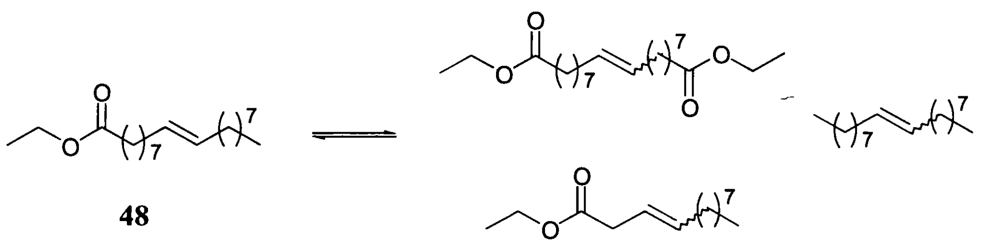
2.3 Olefin Metathesis in Dynamic Combinatorial Chemistry

Cross olefin metathesis has been mentioned[8] as a reversible reaction that may be applied to DCC approach. It has certain advantages over other reactions, most importantly, the low abundance of carbon-carbon double bonds in biomolecules makes cross-reaction with the target less likely. Another useful consequence of this is that it is possible to use reagents in an equimolar ratio that makes analysis easier as the amplification would be more dramatic and consumption of the starting components could also be monitored.

The aim of this part of the project was to apply olefin cross-metathesis of terminal or internal acyclic alkenes (and/or alkynes) in dynamic library formation. Initially, we explored cross-metathesis of internal and terminal acyclic olefins using Grubbs' catalyst to scramble these alkenes and make libraries of compounds without biological target.

2.3.1 Cross-Metathesis of Ethyl Oleate

The first experiment was the cross-metathesis of readily available ethyl oleate in an attempt to demonstrate that the internal alkene of ethyl oleate could be scrambled.



Scheme 2-7: Cross-metathesis of ethyl oleate. Reagents and conditions: i. ethyl oleate, Grubb's catalyst (**18**, 0.01 equivalent), degassed 1,2-dichloroethane, r.t.

This reaction was repeated four times and in the first three times GC-MS showed no scrambling at all. However, in the fourth experiment GC showed seven peaks (Figure 2-4). It was not possible with the analytical tools available at the time, to identify all the peaks, however peaks in the GC-MS did show ethyl oleate, octadecene and diethyl octadecene dicarboxylate (Figure 2-4). We speculate that the scrambling was achieved in this last experiment, but we could not know whether equilibrium was achieved or for how long.

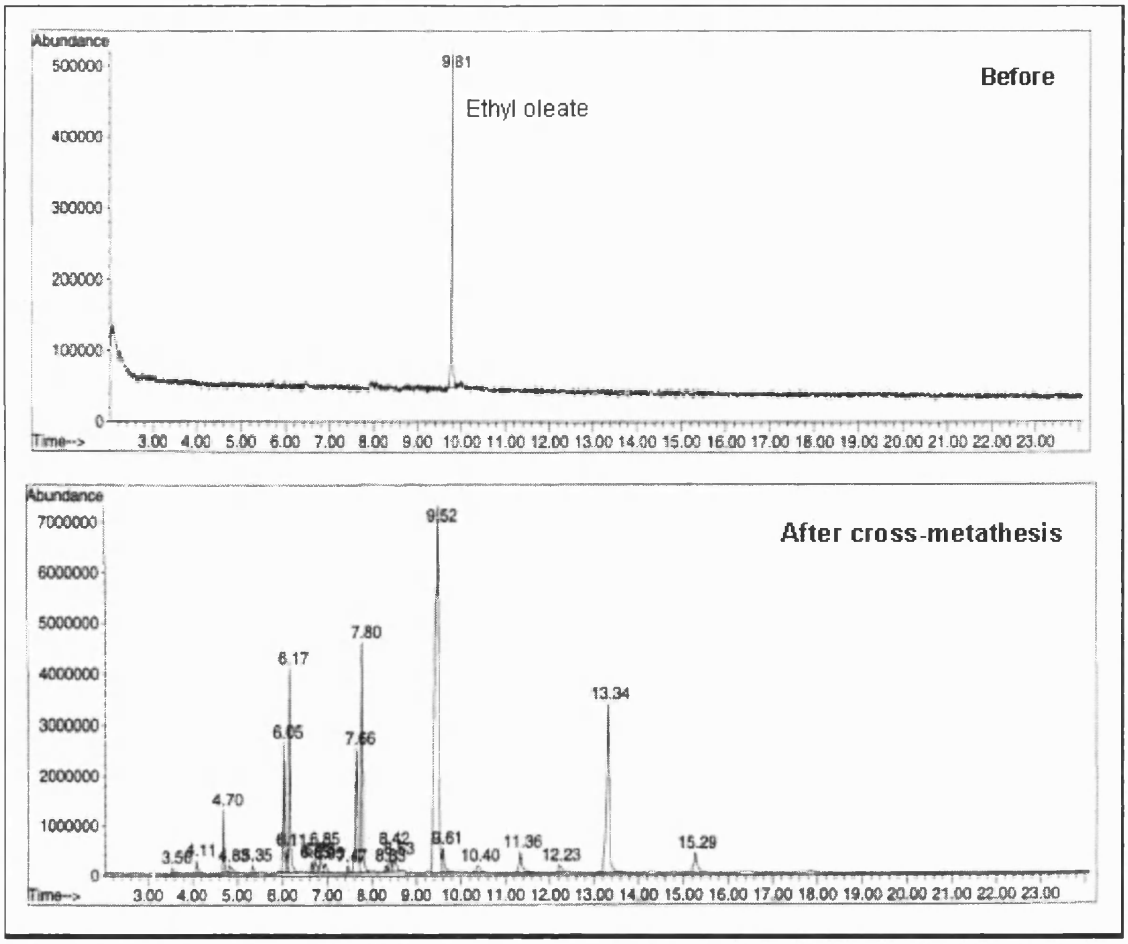
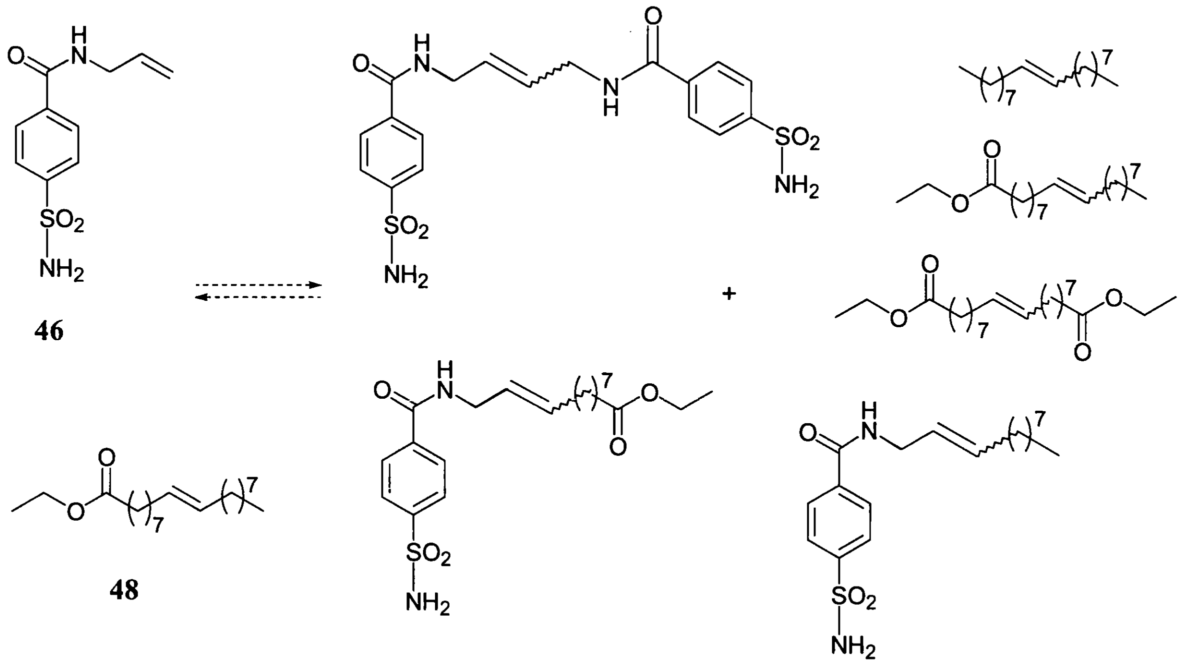


Figure 2-4: GC-MS traces of ethyl oleate and the reaction mixture after cross-metathesis using Grubbs' catalyst **18**.

In the next experiment we attempted to add another compound to the scrambling of ethyl oleate (Scheme 2-8). Unfortunately despite repeated attempts, no scrambling was achieved. We initially speculated that the possible reason for this lack of success was perhaps due to Grubbs' catalyst being very sensitive to air and to the presence of air in the system. Therefore, we envisaged that further study of the reaction and development of stable catalysts would contribute to application of olefin metathesis in DCC. It would also be of interest to attempt dynamic olefin metathesis library creation in water/buffer or other polar solvents. This initiated development of a more robust air-stable polymer-supported catalyst for olefin metathesis and some attempts to make a catalyst that could be used in protic solvents. Work in this area is described in the next chapter.

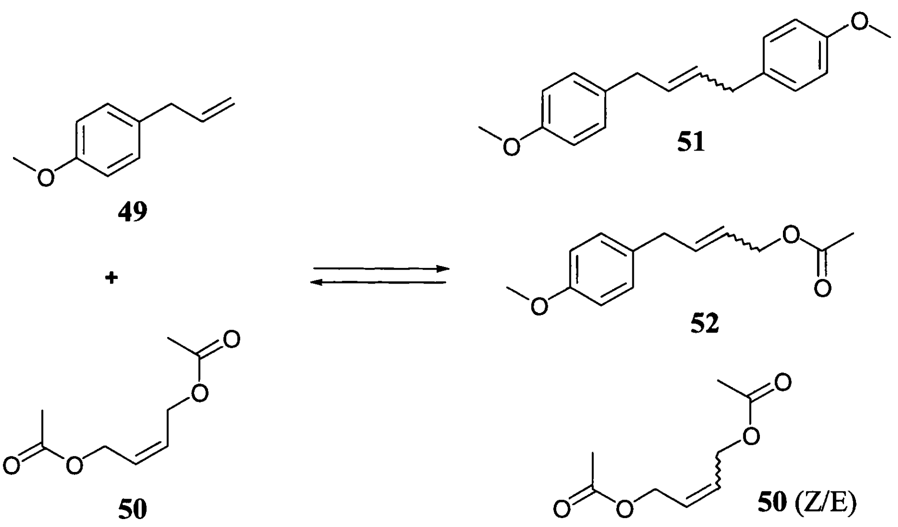


Scheme 2-8: Cross-metathesis library of two starting alkenes. Reagents and conditions: Grubbs' catalyst (**18**), degassed dichloroethane, r.t.

2.3.2 Grubbs' Catalyst in Olefin Cross-metathesis – Effects of Substrate Concentration on Product Distribution and Yield

As our interest in olefin metathesis was initially sparked by the possibility of it being used in target accelerated dynamic library formation, we tried to examine the conditions under which it would be possible to do so. One of the obvious problems is a choice of a suitable solvent, with a tendency to use aqueous conditions whenever possible. There was also a need to use low concentrations (1 mM level or less) of starting library components/substrates for olefin metathesis in order to reduce the quantity of biological target used *in situ* (e.g. enzyme). The main reason for this is the cost of a biological target. This means that a good rate of cross-metathesis reaction is needed at these concentrations. Most commonly Grubbs' catalyst is reported to be used in synthesis at 100 times higher concentrations.

However, Nicolaou *et al.* reported using Grubbs' catalyst in aqueous conditions (using a phase transfer catalyst) to initiate cross coupling of monomeric vancomycin derivatives with terminal alkenes of different carbon chain length in the presence of vancomycin binding targets Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala.[26] They use their target peptides and Grubbs' catalyst at 0.1 mM and substrates (vancomycin monomers) at 0.5 mM concentrations in a two phase system of water : dichloromethane (95:5) with addition of phase transfer catalyst (*N,N,N*-trimethyldodecylammonium-bromide). In the absence of the target expected statistical distribution of products is observed, whereas in the presence of target peptide Ac_2-L-Lys-D-Ala-D-Ala, shorter tethered dimers are formed preferentially (see Chapter 1, Scheme 1-5, page 26).



Scheme 2-9: Cross-metathesis of 4-methoxy-1-(prop-2-enyl)benzene **49** and 1,4-diacetoxybut-2-ene **50**. Reagents and conditions: Grubbs' catalyst 20 mol %, various non-degassed solvents and concentrations (see Table 2-5).

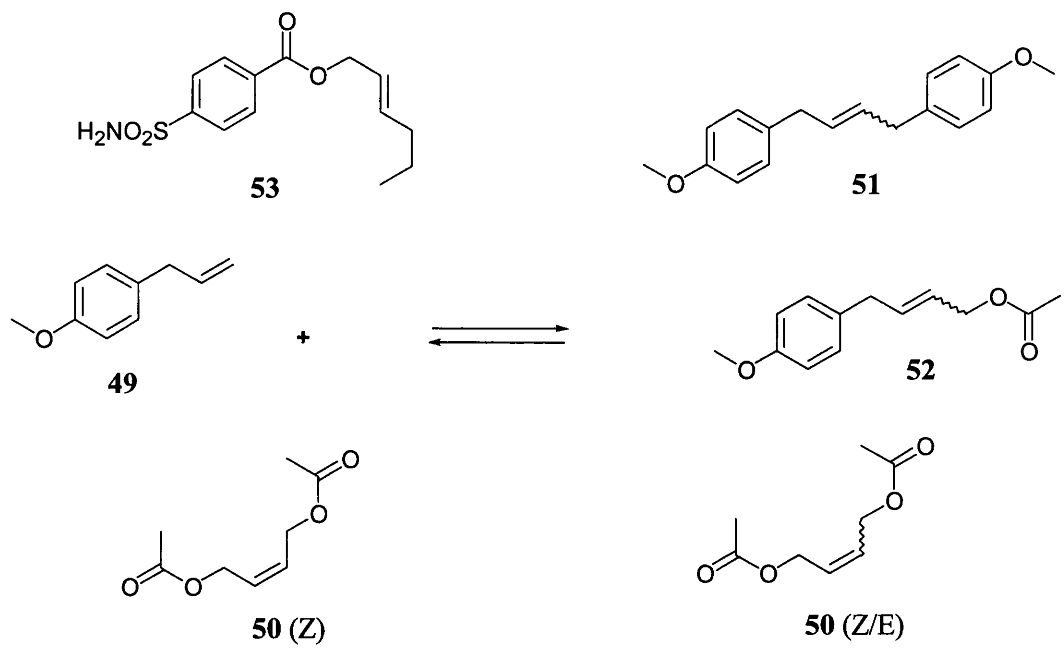
Encouraged by these findings we sought to investigate if it was possible to achieve good rates of cross coupling at low concentrations of substrates first in dichloromethane and toluene and then in two phased systems. We carried out reactions of cross-metathesis of two compounds at series of different concentrations, keeping the catalyst to substrate molar ratio constant at 1:5. Table 2-5 summarises our results.

Table 2-5: Isolated yields of products of cross-metathesis using **18** at different concentrations.

Concentration of **49** (mM)	Volume of solvent (cm^3)	Solvent	Isolated heterodimer **52** (%)	Isolated homodimer **51** (%)	Isolated Z/E DAB[c] **50** (%)
340	1	DCM[d]	76	18	35
170	1	DCM	59	13	-
42	4	DCM	64	13	39
21	8	DCM	45	13	56
10	16	DCM	24	22	51
1	135	DCM	30	33	45
1	135	toluene	33	28	45
1[a]	135	toluene	26	22	45
1[b]	135	tol/water 1:1[b]	23	12	33

[a] Grubbs' catalyst added in three portions of 0.1 equivalent (totalling 0.3 equivalents), [b] toluene/water 1:1 with cetrimide as a phase transfer catalyst (1%), [c] *cis/trans* 1,4-diacetoxy but-2-ene, [d] dichloromethane (non-degassed).

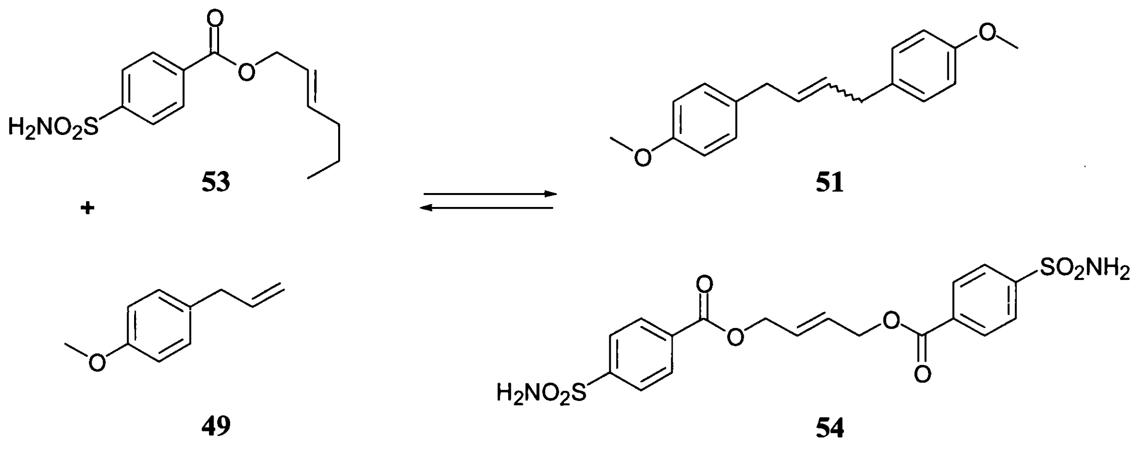
It is clear from the results that the concentration of substrates affects the distribution of products, favouring heterodimer at high concentrations. It is also apparent that the overall yield is lower at low concentrations. Total mass recovery after column chromatography was even lower when a mixture of toluene and water was used in the presence of phase transfer catalyst. This was a disappointing finding, as the reaction does not seem to go to completion at realistic experiment concentrations. We also found that some compounds (e.g. sulfonamide **53**) do not undergo cross- or homo-metathesis at all when Grubbs' catalyst **18** was used as initiator (Scheme 2-10).



Scheme 2-10: Cross-metathesis of three alkenes using catalyst **18**. Reagents and conditions: catalyst **18** (3 x 0.1 equivalent), toluene. Sulfonamide **53** does not react and is recovered unchanged from the reaction mixture (87 %) the other products were isolated by chromatography: heterodimer **52** (30 %), homodimer **51** (11 %) and scrambled (Z/E)-DAB **50** (45 %).

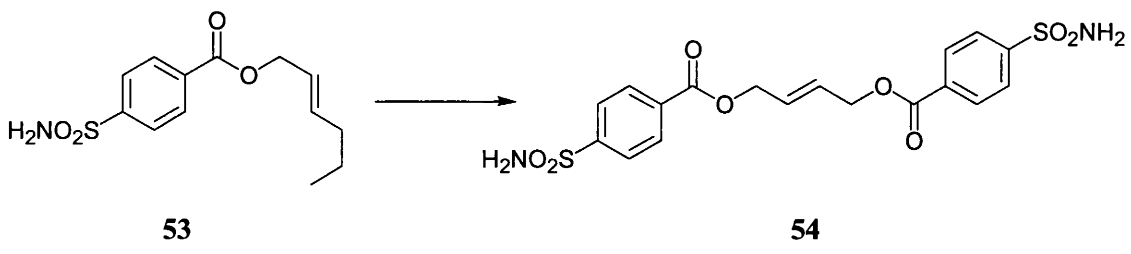
Attempts were also made to include a third component in this scrambling experiment. Sulfonamide **46** was poorly soluble in dichloromethane or toluene, so a new sulfonamide **53** was prepared (Chapter 3, Scheme 3-4) that was readily soluble in dichloromethane and sufficiently soluble in toluene. Cross-metathesis reaction of **49** and **50** was carried out in the presence of sulfonamide **53** using Grubbs' catalyst **18** and this resulted in scrambling of **49** and **50** whereas sulfonamide **53** was recovered from the reaction unchanged (Scheme 2-10). When second generation catalyst **20** was used to initiate cross-metathesis of sulfonamide **53** and 4-methoxy-1-(prop-2-enyl)benzene **49**, sulfonamide dimer **54** precipitated out from the solution within minutes and was isolated by filtration (66 %) and the homodimer **51** was isolated (58 %) using column chromatography of the remaining filtrate (Scheme 2-11). Homo-coupling of sulfonamide **53** was repeated (Scheme 2-12) using catalyst **20** and the sulfonamide-dimer **54** again precipitated within minutes and was isolated by filtration in 74 % yield.

This homo-coupling was also attempted using Grubbs' catalyst **18**, but only unchanged starting material was detected in the reaction mixture.



Scheme 2-11: Cross-metathesis of 4-methoxy-allylbenzene **49** and sulfonamide **53** using catalyst **20**. Reagents and conditions: catalyst **20** (0.1 equivalent), dichloromethane, 40 ˚C, 6 h. Sulfonamide dimer **54** crashed out of the reaction mixture and was isolated by filtration (66 %), estragole homodimer **51** (58 %) and unreacted **49** (29 %) were isolated by chromatography.

It seems that, in contrast with Grubbs' catalyst **18**, second-generation initiator **20** can effect olefin metathesis of these sulfonamides, but the only product seems to be the virtually insoluble sulfonamide-dimer. In this case the sulfonamide dimer **54** is effectively sequestered from the equilibrium, as insoluble precipitate, thus driving the equilibrium toward further production of dimer **54**. This creates a reaction bias, which is a major obstacle for using of olefin metathesis as means of creating a library of sulfonamides as carbonic anhydrase inhibitor candidates. However, it is not clear whether precipitation occurs under aqueous conditions and these results do not preclude the use of cross-metathesis for production of other DCLs.



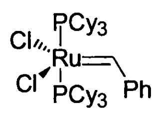
Scheme 2-12: Homo-coupling of sulfonamide **53**. Reagents and conditions: catalyst **20** (0.05 equivalent), dichloromethane, 40 °C, 30 min. Sulfonamide dimer **54** crashed out of the reaction mixture within minutes and was isolated by filtration (74 %).

After initial efforts to employ olefin metathesis in the generation of the dynamic library of CA inhibitor candidates, we were under the impression that the problems associated with it were due to the poor activity and sensitivity of catalyst **18** as well as the solubility and cross-metathesis reactivity of the selected substrates. We thought that some of these obstacles would be avoided by development of a range of suitable olefin-metathesis initiators that could operate in a range of conditions. These developments are described in the following chapter.

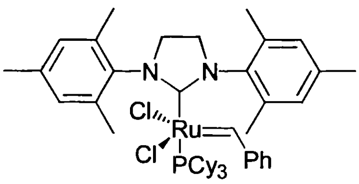
3 POLYMER-SUPPORTED ALKYLIDENE RUTHENIUM INITIATORS FOR OLEFIN METATHESIS – RESULTS AND DISCUSSION

3.1 Development of Air-Stable Polymer-Supported Initiator for Olefin Metathesis – 'the First Generation'

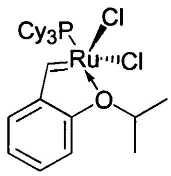
The possibility of adapting the olefin-metathesis initiator to better suit the requirements of dynamic combinatorial chemistry (DCC) was explored. For dynamic diversity experiments, any initiator that reduces ruthenium contamination, bypasses the need for use of degassed solvents, or simplifies handling is desirable. To address this, development of a robust immobilised initiator for olefin metathesis that can be modified to operate in a range of solvents and in a normal atmospheric environment was pursued.



18



20



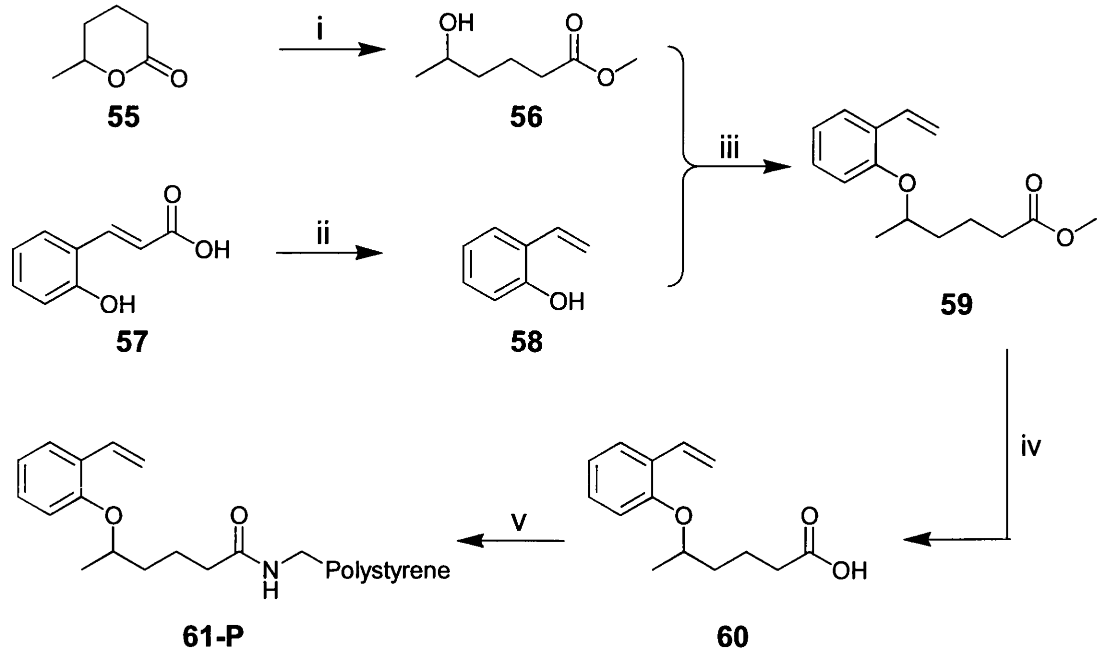
21

The stability of the ruthenium chelate **21** (see Chapter 1, page 39-40, Figure 1-11)[65,66] was attractive as it could be recovered from the reaction mixture by column chromatography using non-degassed reagent grade solvents and reused in a subsequent reaction with minimal loss of activity. The isopropyl group was reported to be essential for activity and stability of **21**, due to its steric effects. We thought that extending one side of the isopropyl group of the phenol ether in **21** and introduction of a suitable functional group would allow attachment to any chosen support. Aminomethyl polystyrene was selected as a solid support as it was reasonably priced, had good swelling properties in dichloromethane and attachment of a suitable carboxylic acid ligand could be achieved following standard coupling procedures for solid supported reagents (Scheme 3-1).

3.1.1 Synthesis of the 'First Generation' Polymer-Supported Catalyst

We initially opted for the quick, rather than efficient, route that would afford ligand **60** in just four steps so that the concept could be quickly tested and changes in ligand design introduced, if necessary, before further optimisation of the synthetic route was pursued. Racemic δ-hexanolactone **55** was opened with sodium methoxide in methanol to afford methyl 5-hydroxyhexanoate **56**[96] in 63% yield. 2'-Hydroxy-cinnamic acid was decarboxylated using Kugelrohr apparatus, previously lined with 1,4-benzoquinone to prevent polymerisation of the generated 2-vinylphenol.[97] 2-Vinylphenol is not only prone to polymerisation, but also very toxic, so it was promptly used crude without further purification, as a mixture with *p*-benzoquinone, in subsequent Mitsunobu coupling. In spite of the use of polymerisation inhibitor and other measures this reaction was a disappointment as only several, out of many, attempts afforded rather low yield of 2-vinylphenol (Scheme 3-1).

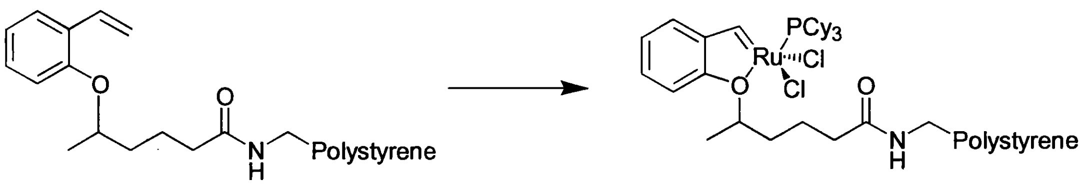
Misunobu reaction was chosen as the next step, as it was perceived to be the fastest route to the desired ligand. Crude 2-vinylphenol and methyl 5-hydroxyhexanoate were coupled in the presence of triphenylphosphine and diisopropyl azodicarboxylate in tetrahydrofuran. After column chromatography methyl 5-(2-vinylphenyloxy)hexanoate **59** was isolated in 20% yield (over two steps, based on 2-hydroxycinnamic acid). Hydrolysis of **59** with aqueous NaOH in 1,4-dioxane provided acid **60** in 77% yield (Scheme 3-1).



Scheme 3-1: Synthesis of the supported ligand **61-P**. Reagents and conditions: i. sodium methoxide, methanol, 0 to 25 °C, 63%; ii. 210 °C; iii. Diisopropyl azodicarboxylate, triphenylphosphine, tetrahydrofuran, r.t., 20%; iv. aqueous sodium hydroxide, 1,4-dioxane, 77%; v. aminomethylpolystyrene, diisopropylcarbodiimide, 1-hydroxybenzotriazole, dimethylformamide: dichloromethane (1:1), r.t.

Reacting the acid **60** with amino-methyl-polystyrene in the presence of diisopropylcarbodiimide afforded the polystyrene-supported alkene **61-P** which was treated with an equimolar amount of Grubbs' catalyst **18** for 2 h at room temperature. Filtration and washing of the resin gave **62-P** as dark brown beads that could be dried, stored in air and used more than a month later with no apparent decrease in activity. This first batch of resin loaded with equimolar amount of Grubbs' catalyst resulted with a resin of low loading (0.12 $mmolg^{-1}$, as determined by phosphorus analysis). We suspect that this is due to liberated free phosphine, which is reported[58,59] to inhibit olefin metathesis by reassociation with the active species. Therefore, in future attempts an alternative method of loading the resin five (or more) times with 10 mol% of Grubbs' catalyst was adopted instead (Scheme 3-2). The first resin made this way was also analysed for phosphorus content and its loading had practically doubled (0.20 $mmolg^{-1}$).

None of the subsequent batches were subjected to elemental phosphorus analysis due to its cost (approximately £100 per sample).



61-P **62-P**

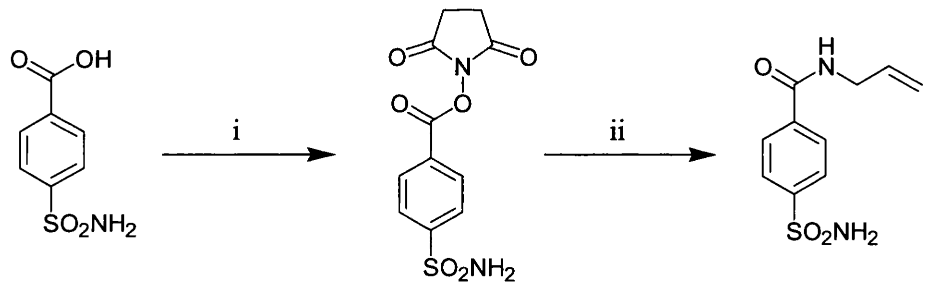
Scheme 3-2: Synthesis of polymer-supported catalyst **62-P**. Reagents and conditions: Grubbs' catalyst (**18**) 5 x 0.1 equivalent, degassed 1,2-dichloroethane, r.t.

Loading of acetylated aminomethyl-polystyrene with Grubbs' catalyst **18** was also attempted in order to show whether there is exchange between alkylidine ruthenium of Grubbs' catalyst and the vinyl residue of our polymer bound ligand, rather than the vinyl residues of polystyrene itself or non specific adsorption of Grubbs' catalyst onto polystyrene. Aminomethyl-polystyrene was acetylated with acetic anhydride to give *N-capped polystyrene*, then agitated with equimolar quantity of Grubbs' catalyst to afford brown resin and tested for ring-closing metathesis of **69a** under standard conditions (see experimental). ^{1}H NMR integration suggests only 18 % conversion to cyclic product and there was no conversion when recycling was attempted. This indicates that, to some extent, the reaction with the vinyl residues or non-specific adsorption of the **18** probably takes place, although this is not sufficient to account for the observed activity, or more importantly, the recyclability of the supported initiator **62-P** (testing results, page 82).

3.1.2 Synthesis of Substrates for Olefin Metathesis

Activity of olefin-metathesis catalysts is conveniently tested by ring-closure of suitable diene substrates to afford 5- to 6-membered carbo- or hetero-cyclic rings. The catalyst's ability to initiate reversible cross coupling of two alkenes can be tested using equimolar mixtures of two terminal or/and internal alkenes as substrates. Several compounds were synthesised as substrates for testing of the resin bound initiators. Syntheses of compounds intended for use as starting components for dynamic library formation using reversible cross-olefin metathesis are also presented here.

Some of the compounds were initially selected and made as building blocks for testing the dynamic combinatorial strategy using olefin metathesis. Several compounds were made with sulfonamide and an alkene functionality and several others with alkene functionality principally in order to act as building blocks for assembly of libraries of potential carbonic anhydrase inhibitors in DCC approach. Problems experienced achieving cross-coupling of these compounds with Grubbs' catalyst **18** initiated the development of polymer-supported initiators for olefin metathesis (Chapter 2, Schemes 2-8 to 2-12, pages 68-73). Other substrates, mostly dienes were specifically selected and synthesised to be used as substrates for testing ring-closing activity of our polymer-supported initiators.

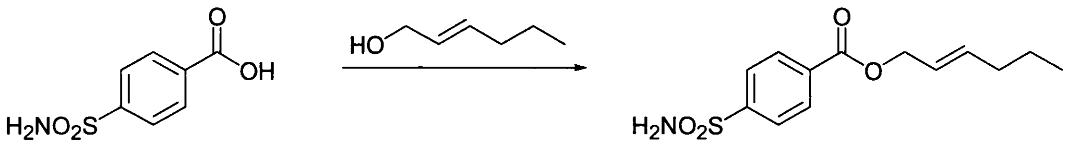


44 **63** **46**

Scheme 3-3: Synthesis of **46**. Reagents and conditions: i. *N*-hydroxysuccinimide, 4-dimethylaminopyridine, dicyclohexylcarbodiimide, r.t., ii. allylamine, tetrahydrofuran, r.t., 56%.

Allyl amide **46** was made in two steps starting with 4-sulfamoylbenzoic acid **44** and activated as the *N*-hydroxysuccinimide ester (**63**, Scheme 3-3). Analysis of the crude using ^{1}H NMR showed material to be sufficiently pure and free of dicyclohexylurea to be used for further synthesis, without purification. Thus, direct reaction of **63** with allylamine in tetrahydrofuran at room temperature afforded **46** in 56% yield.

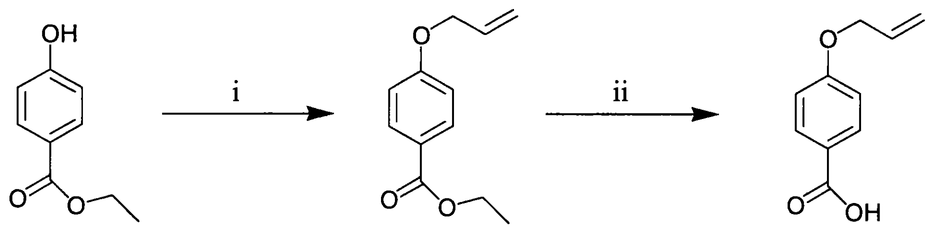
As sulfonamide **46** was poorly soluble in dichloromethane, toluene and other organic solvents that are commonly used for olefin metathesis, an alternative similar compound with more lipophilic properties was needed, so ester **53** was synthesised from hex-2-en-1-ol. Direct coupling of 4-sulfamoylbenzoic acid and hex-2-en-1-ol in the presence of 4-dimethylaminopyridine and dicyclohexylcarbodiimide afforded hex-2-enyl 4-sulfamoylbenzoate **53** in a poor 29 % yield (Scheme 3-4). However, even this poor yield afforded sufficient quantities of **53**, so there was no need to repeat the reaction.



44 **53**

Scheme 3-4: Synthesis of hex-2-enyl 4-sulfamoyl-benzoate **53**. Reagents and conditions: 4-dimethylamino pyridine, dicyclohexylcarbodiimide, dimethylformamide, r.t., 20h, 29 %.

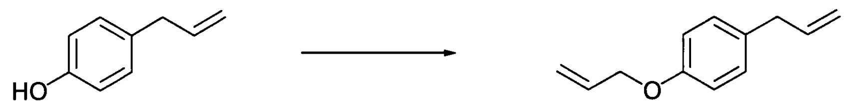
Ethyl 4-allyloxybenzoate **64** was synthesised from ethyl 4-hydroxybenzoate by treatment with allyl bromide and NaH in dimethylformamide for 60 h at room temperature in 84% yield (Scheme 3-5). Compound **64** was then hydrolysed with KOH and water in tetrahydrofuran to cleave the ester, affording acid **47** as white crystals in 42% yield.



64 **47**

Scheme 3-5: Synthesis of 4-allyloxybenzoic acid **47**. Reagents and conditions: i. Allyl bromide, NaH in dimethylformamide, 0 to 25 °C, 84%; ii. NaOH in 1,4-dioxane, r.t., 42%.

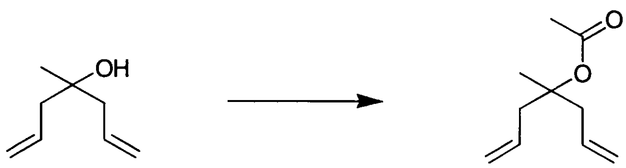
4-Allyloxyallylbenzene **65** was synthesised by reacting 4-allylphenol with allyl bromide under basic conditions. Distillation under reduced pressure afforded ether **65** in 96 % yield (Scheme 3-6).



65

Scheme 3-6: Synthesis of 4-allyloxyallylbenzene **65**. Reagents and conditions: potassium carbonate, allyl bromide, acetone, r.t, 96 h, 96 %.

4-Methylhepta-1,6-dien-4-yl acetate **67a** was obtained by acetylation of 4-methylhepta-1,6-diene-4-ol **66a** in pyridine. Flash chromatography afforded **67a** as a colourless liquid in 56 % (Scheme 3-7).

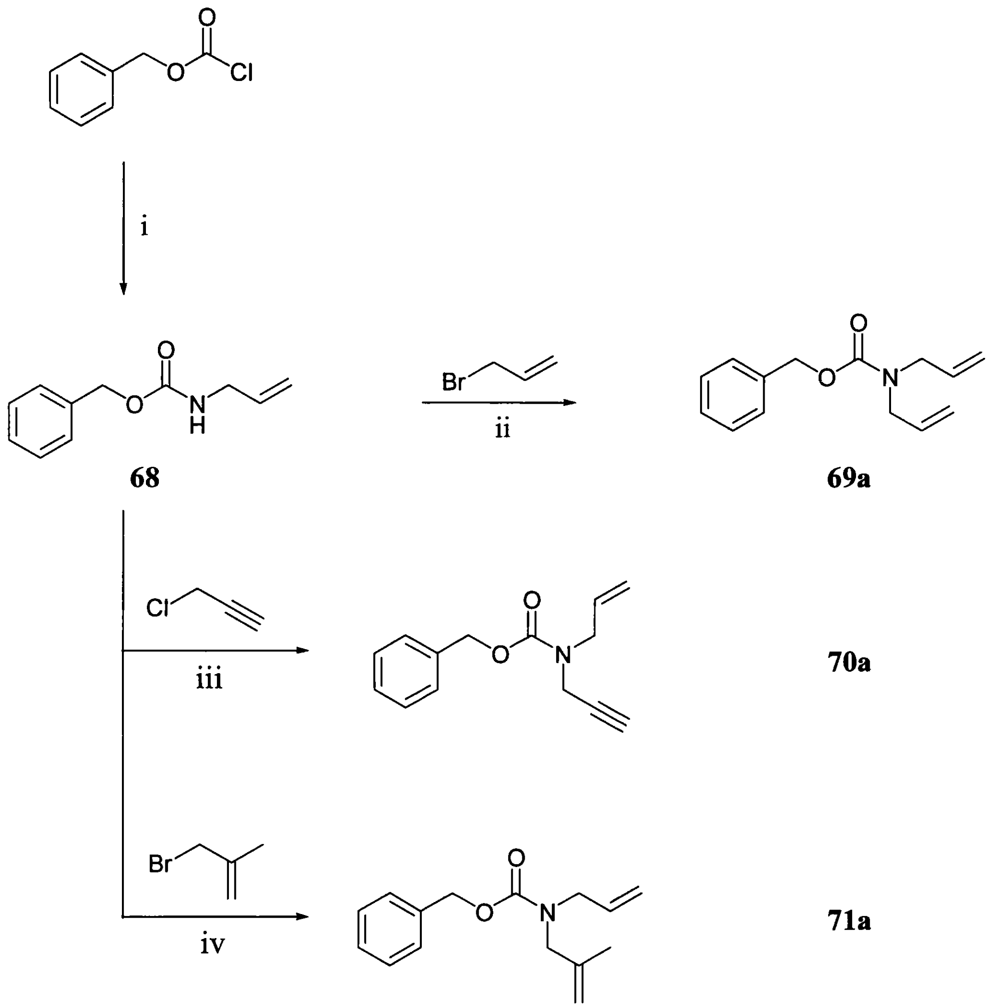


66a **67a**

Scheme 3-7: Acetylation of 4-methylhepta-1,6-dien-4-ol. Reagents and conditions: i. Acetic anhydride in pyridine, 60 °C, 56 %.

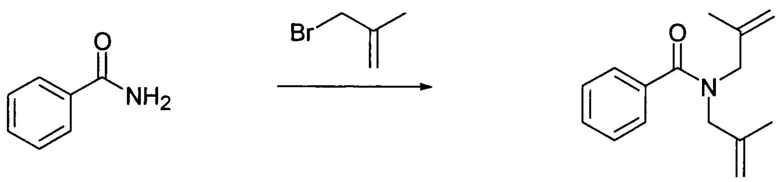
Benzyl N-allylcarbamate **68** was made from benzyl chloroformate and allylamine in 99% yield. Cbz-allylamine **68** was treated with allyl bromide in the presence of sodium hydride to give *benzyl N,N-diallylcarbamate* **69a** in 99 % yield (Scheme 3-8), which was later used to test the activity of polymer-supported catalyst for olefin metathesis.

We were interested to see if our second generation initiator **90-P** (Scheme 3-17, page 95) could effect metathesis of substituted alkenes and en-yne metathesis, so synthesis of suitable alkenes **70a** and **71a** was pursued for this purpose. Benzyl *N*-allylcarbamate **68** was first deprotonated with sodium hydride and then alkylated with propargyl chloride, followed by purification by column chromatography to afford *N-allyl-N-(prop-2-ynyl) benzyl carbamate* **70a.** Similar alkylation of **68** with 3-bromo-2-methylpropene afforded *benzyl N-allyl-N-(2-methylprop-2-enyl) carbamate* **71a** in 87 % yield after purification by flash chromatography (Scheme 3-8).



Scheme 3-8: Synthesis of substrates **69a, 70a** and **71a**. Reagents and conditions: i. Allylamine, r.t., 99%; ii. allylbromide, NaH, 0 to 25 °C, 99%; iii. NaH, dimethylformamide, 0 to 20 °C, 24 h, 87 %; iv. NaH, dimethylformamide, 0 to 20 °C, 24 h, 65 %.

Testing initiator **90-P** for RCM of tetrasubstituted dienes was also desirable, so **72a** was made for this purpose in one step from benzamide. To make *N,N-bis-(2-methyl-prop-2-enyl)benzamide* **72a**, benzamide was treated with sodium hydride followed by excess of 3-bromo-2-methylpropene. Work-up and purification by chromatography yielded 64 % of amide **72a** (Scheme 3-8).



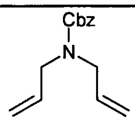
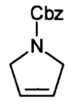
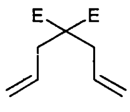
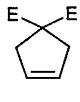
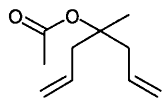
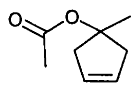
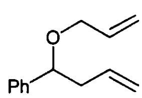
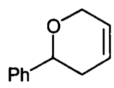
72a

Scheme 3-9: Synthesis of *N,N*-bis(2-methylprop-2-enyl)benzamide **72a**. Reagents and conditions: NaH, dimethylformamide, 0 to 20 ˚C, 64 %.

3.1.3 Testing of the First Generation Polystyrene-Supported Initiator for Olefin Metathesis

Polymer-supported complex **62-P** was then tested for activity using α,ω-diene compounds for ring-closing metathesis (table 1). It is notable that the rates of reaction were slower than those reported using homogenous Grubb's alkylidene **18**, for example reaction of diethyl diallylmalonate, a commonly used substrate for RCM (entry 2) was only 42% complete after 90 min. All of these reactions however, provided good to quantitative yield of product within five hours (Table 3-1). Preparative scale transformation (5 mol% **62-P**, 4h) of benzyl *N,N*-diallylcarbamate gave isolated product in 91 % yield after chromatography.

Table 3-1: Olefin metathesis using polymer-supported complex **62-P** [a]

Entry	Substrate [b]	Product	% Conversion[c] after 90 min	% Conversion[c] after 5 h
1	 **69a**	 **69b**	91	95
2	 **73a**	 **73b**	43	88 [d]
3	 **67a**	 **67b**	77	95 [e]
4			>95	>95

[a] All entries performed with 25 mg of substrate and 25 mg of **62-P** (5 mol%) in non-degassed dichloromethane, r.t., 90 min and five hours; [b] Cbz = benzyloxycarbonyl, E = CO_2Et; [c] Relative integration of ^1H NMR; [d,e] overnight runs (17 h) afforded [d] 91 % and [e] 100 % conversion (^1H NMR integration).

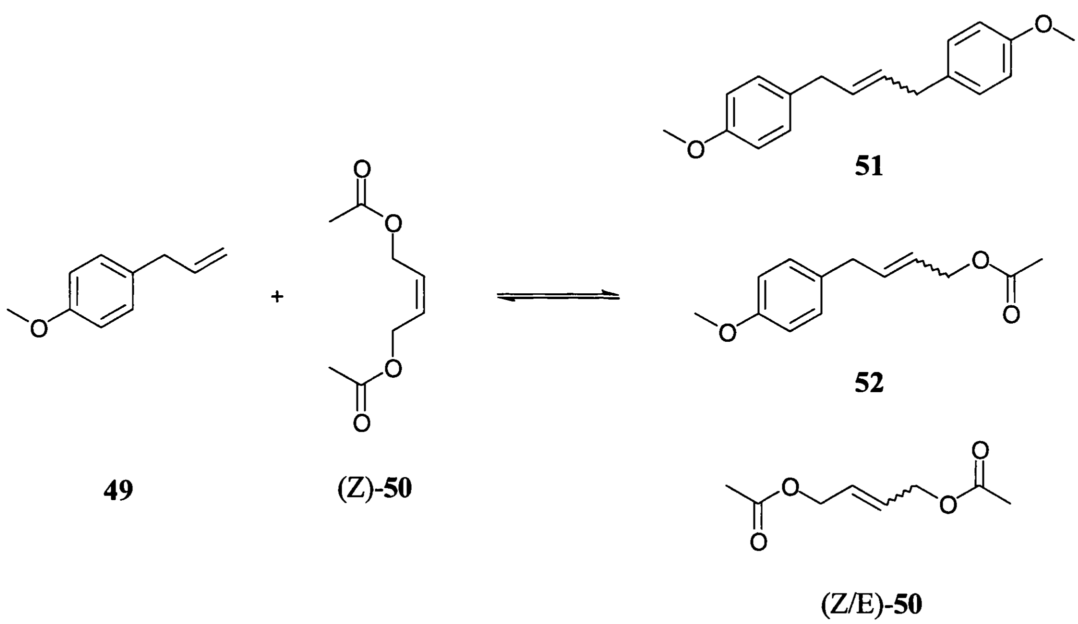
Recycling of **62-P** was tested by treatment of the same batch of resin with equal, successive portions of benzyl *N,N*-diallylcarbamate, without addition of stabilising alkene (Table 3-2).[78] Indeed, **62-P** proved to be remarkably robust. Early experiments using a particularly low loading of 1.5 mol% of **62-P** (0.12 mmolg^{-1}) provided good yields for the first three runs, followed by a rapid decline in activity in the subsequent three runs, during which time the beads turned from dark brown to black. When a higher catalytic loading of approximately 5 mol % (0.20 mmolg^{-1}) of catalyst was used good yields were obtained over five successive runs (Table 3-2) and the beads remained active and dark brown for longer.

Table 3-2: Recycling of **62-P** for the RCM of benzyl-*N,N*-diallylcarbamate (**69a**) [a]

Conversion% [b]						
1	**2**	**3**	**4**	**5**	**6**	**7**
91 [c]	81	68	67	63	46	40
81 [d]	69	68	33	21	11	-

[a] All recycling runs performed with the same portion of resin, each time with the new portion of substrate **69a**, in non-degassed dichloromethane for 90 minutes; [b] Relative integration of ^{1}H NMR; [c] 5 mol% **62-P**; [d] 1.5 mol% **62-P**.

The ability of **62-P** to perform cross-metathesis was also demonstrated (Scheme 3-10). Heating (Z)-1,4-diacetoxy-but-2-ene (Z-**50**) with 4-allyl-anisole (**49**) in a 2:1 ratio and **62-P** in dichloromethane under reflux for 9 h afforded the cross-coupled product **52** (33 %) along with the homodimer **51** (18 %). The recovered resin retained its brown colouration, rather than turning black. Subsequent ring-closing metathesis of benzyl *N,N*-diallylcarbamate **69a** using this same batch of resin afforded 85 % conversion (^{1}H NMR integration) of the cyclised product over 90 min, confirming the retention of the catalytic activity.



Scheme 3-10: Testing for cross-metathesis activity of supported initiator **62-P**. Reagents and conditions: 25 mg of **49**, 56 mg of (Z)-**50** and 25 mg of resin **62-P**, dichloromethane, reflux, 9 h; isolated yields after column chromatography: **51** 33 %, **52** 18 % (based on **49**).

Testing results show **62-P** to be robust enough to use in non-degassed solvents and to store in normal atmospheric conditions. The initiator **62-P** is recyclable in up to five cycles of RCM without the addition of additives and has also performed well in cross-metathesis of suitable substrates.

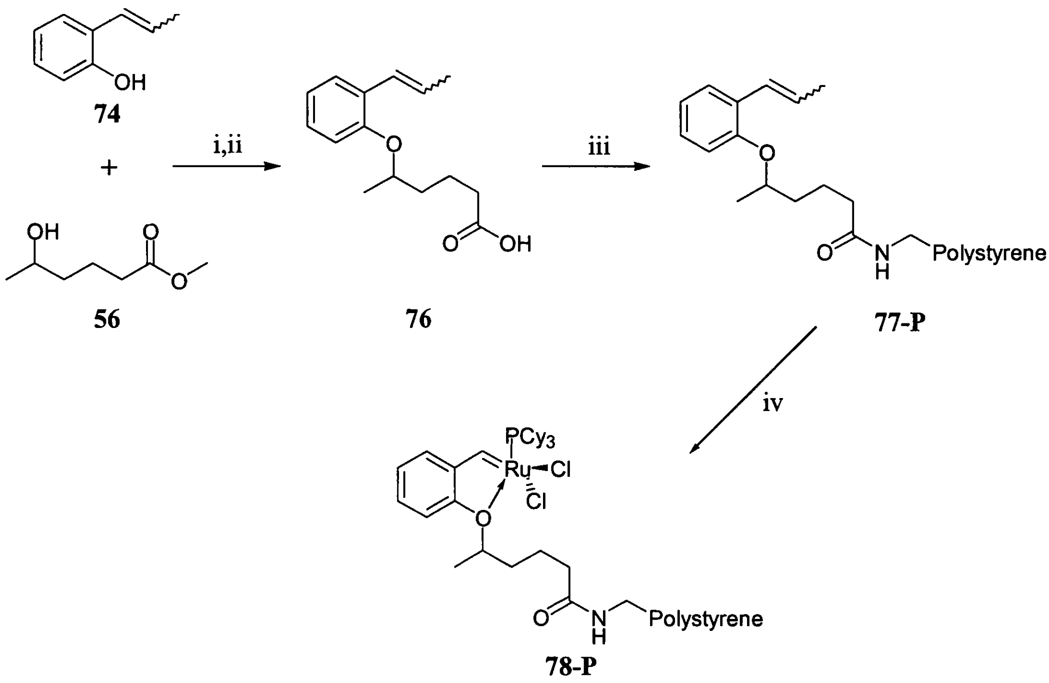
That our initiator could be repeatedly used in non-degassed solvents was satisfying as olefin metathesis initiated with Grubbs' catalyst **18** is typically reported in degassed solvent. We decided to check and were surprised to find that test compound **69a** was effectively converted to the closed compound in 90 minutes by catalyst **18**. It is not clear how long Grubbs' catalyst **18** remains active in the presence of more challenging substrates.

3.1.4 Alternative Route to the Polymer-Supported Initiator

Initiator **62-P** is robust and recyclable, but the rapid synthesis although fast, was very unreliable and gave disappointing yields. Particularly problematic was the synthesis of 2-vinylphenol **58**, which is prone to polymerisation apparently irrespective of the presence of benzophenone as an inhibitor and is also toxic.

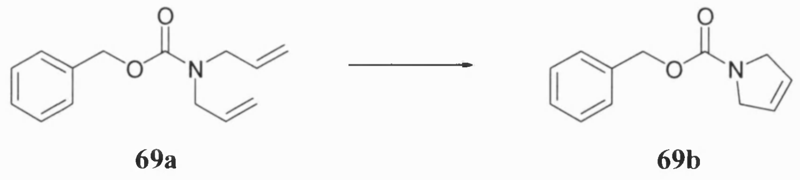
We reasoned that it would be possible to replace 2-vinylphenol as a starting material with commercially available 2-(prop-2-enyl)phenol (**74**), a simple replacement of a terminal alkene with an internal/substituted one.

Racemic methyl 5-hydroxy hexanoate **56** was thus coupled to 2-(prop-2-enyl)phenol **74** under Mitsunobu conditions to afford a mixture of *cis* and *trans* (approximately 20 % *cis* to 80 % *trans*) methyl-5-(2-(prop-2-enyl)phenoxy)hexanoate **75** in 53 % yield. Subsequent hydrolysis of the ester with aqueous sodium hydroxide gave the acid **76** in 86 % yield. Following the same procedure as before, the acid was then coupled onto the amino-methyl polystyrene to obtain polystyrene-ligand **77-P** and the ligand was then loaded by repeatedly reacting with 10 mol % Grubbs' catalyst five or more times to give a polystyrene-supported initiator **78-P** (Scheme 3-11).



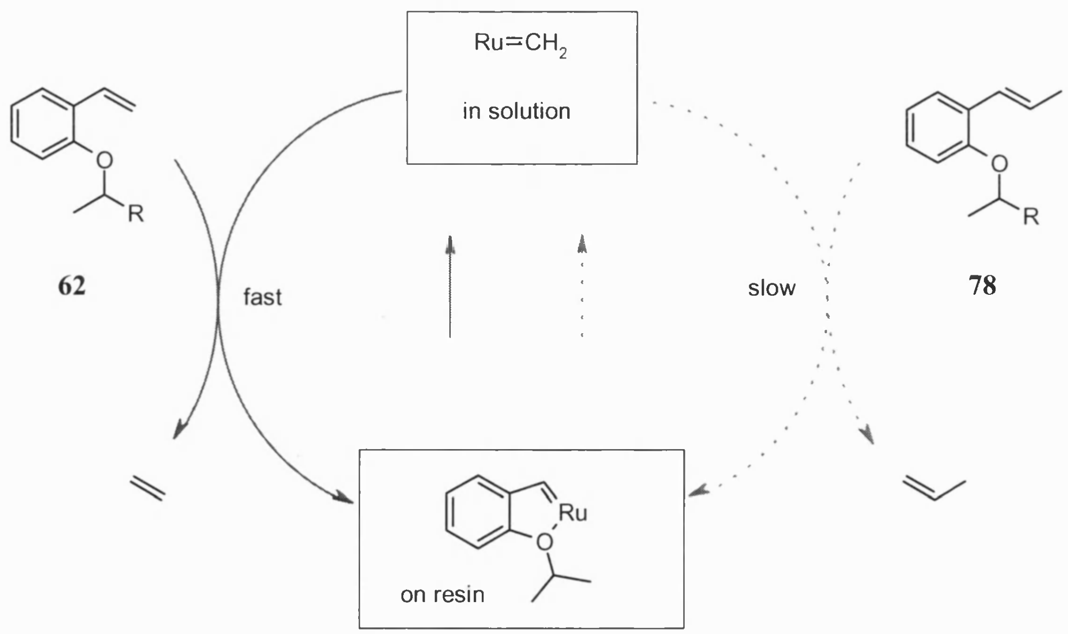
Scheme 3-11: Synthesis of polystyrene-supported alkylideneruthenium **78-P** starting from **74**. Reagents and conditions: i. Diisopropyl azodicarboxylate, triphenylphosphine, tetrahydrofuran, r.t., 53 %; ii. aqueous sodium hydroxide, 1,4-dioxane, 86 %; iii. diisopropylcarbodiimide, 1-hydroxybenzotriazole, dimethylformamide : dichloromethane (1:1), r.t.; ii. Grubbs' catalyst (**18**) 5 x 0.1 equivalent, degassed 1,2-dichloroethane, r.t.

The initiator was tested following the standard procedure using benzyl *N,N*-diallylcarbamate **69a** as substrate (Scheme 3-12). Conversion rates (as determined by ^{1}H NMR integration) ranged from 63 to 85 % in the first run and only 21 % in the second cycle. Ring-closing activity was also tested in toluene and was comparable to that found in dichloromethane (77 %). It was disappointing that this initiator **78-P** was not as active as the original **62-P**, as it gave slightly lower yields for ring-closing metathesis and there was no significant recycling.



Scheme 3-12: Ring-closing metathesis of Cbz-diallylamine **69a** initiated by initiator **78-P.**

A possible explanation for poor recycling of **78-P** compared to **62-P** follows. We know that saturation of the solid supported ligand (vinyl for **62-P**, alkenyl for **78-P**) does not occur (only five times 10 mol% of **18** is loaded onto the ligand), presumably leaving 'vacant' ligand sites (vinyl for **62-P**, alkenyl for **78-P**). In the case of RCM of **69a** the methylidene ruthenium 'Ru=CH_2' (Scheme 3-13) is the only catalytic/propagating species and has a relatively short half-life of approximately thirty minutes in dichloromethane. For successful recycling, this species must be promptly intercepted by the chelate when all the substrate has been used up. More rapid reaction seems to occur with the vacant vinyl ligands, compared to the alkenyl ligands, leading to a difference in the recycling efficiency.



Scheme 3-13: Catalytic cycle of a 'boomerang' catalyst.

Further optimisation of the synthetic route to the vinyl ligand **60** is currently being pursued by our group (Regourd).

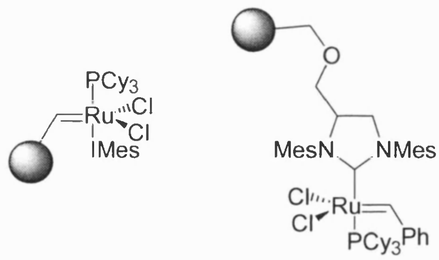
3.1.4.1 The use of phosphine scavengers in preparation of polymer-supported initiators

As previously discussed, loading of the polystyrene bound ligand was carried out by multiple loading of 10 mol% of Grubb's catalyst **18** in order to avoid accumulation of the free phosphine that inhibits further reaction. This procedure can be tedious and it would be advantageous if an alternative solution to a problem of accumulating free phosphine was found that would allow the loading to be carried out in one go. For this reason, an attempt was made to improve loading of the Grubbs' catalyst onto the polymer-supported ligand **77-P** by addition of a phosphine scavenger copper (I) chloride, which is known to increase the rate of olefin metathesis by binding the free phosphine liberated during the reaction into an ill-defined complex.[58] One equivalent of solid CuCl was added to the reaction vessel, together with one equivalent of Grubbs' catalyst and agitated with the polymer-supported ligand **77-P** in dichloromethane for two hours at room temperature. Filtration and washing afforded a dark brown to black resin, that was then tested for RCM activity using standard procedure with benzyl *N,N*-diallylcarbamate **69a**.

Various batches of resin obtained in this way gave conversion of **69a** into product **69b** ranging from 32 to 65 % in the first run and attempts to recycle the resin were unsuccessful. This lead to a decision to abandon the use of CuCl as a phosphine scavenger in the preparation of supported initiators. Although successful use of CuCl has been reported in synthesis of homogenous catalysts[70,72] and even in a preparation of a polymer-supported initiator **92** (Figure 3-3, page 101),[98] it is possible that residues of the poorly soluble copper-phosphine complex or CuCl remain on the resin after washing and affect its activity in subsequent testing experiments.

3.2 Second Generation Phosphine-Free Polymer-Supported Alkylidene Ruthenium

The introduction of a new generation of ruthenium-based olefin-metathesis initiators coordinated with 1,3-dimesitylimidazol-2-ylidene (IMes) and particularly 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene (SIMes) ligands[57] in 1999 (see Chapter 1, Figure 1-9, page 35) was soon followed by development of second generation polymer-supported and macromolecular initiators bearing these ligands. The first reported polymer-supported initiator bearing NHC ligand was Barret's second generation 'boomerang' polystyrene-supported initiator **35** (Figure 3-1, also see Chapter 1).[80]



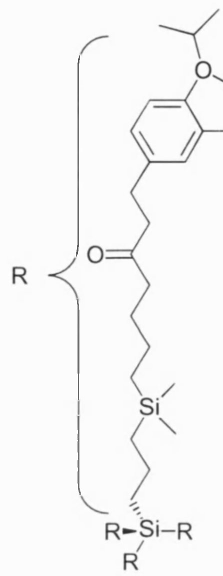
35 **79**

Figure 3-1: Some polymer-supported catalysts bearing Arduengo carbene ligands.

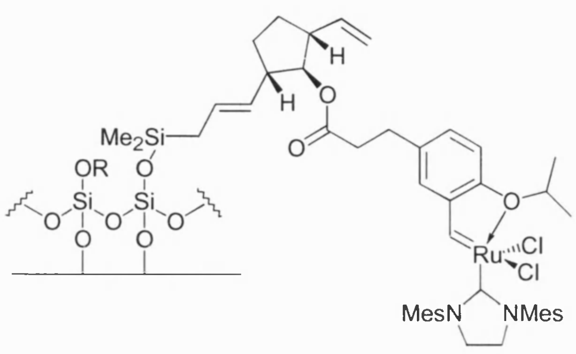
Further developments in the area soon followed. Blechert's[99] Merrifield-polystyrene-supported second-generation initiator **79** (Figure 3-1) performed well in RCM, cross-metathesis and yne-ene metathesis and was recyclable.

Hoveyda's group prepared two macromolecular dendritic Ru-initiators **80** and **81**.[67] Isolation of the product required a very simple silica gel chromatography, as dendrimers are very polar and therefore easy to separate from products. Initiators **80** and **81** (Figure

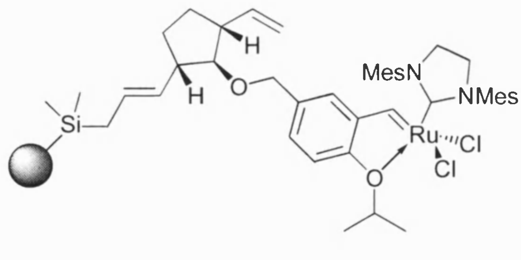
3-2) had activity comparable to that of their parent monomers **21** and **22** (Figure 1-11, page 40). The experiences from the study of dendrimeric initiators were used in design of the glass supported initiators **82**, **83** and **84** (Figure 3-2) by the same group.[100] These catalysts showed good activity and minimal contamination of the isolated product, along with easy handling.



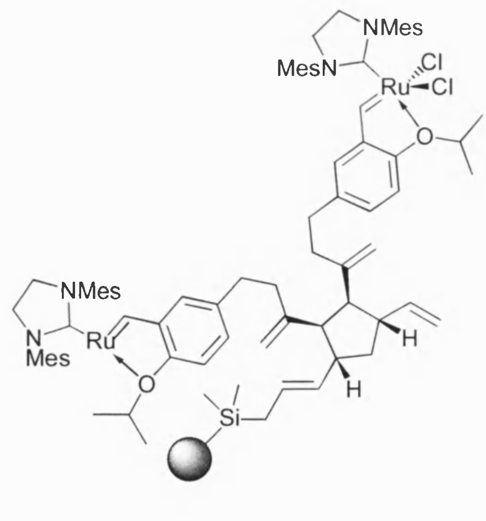
80 L = PCy_3
81 L = SIMes



82



83



84

Figure 3-2: Hoveyda's dendritic-macromolecular and glass-supported initiators bearing NHC ligands.

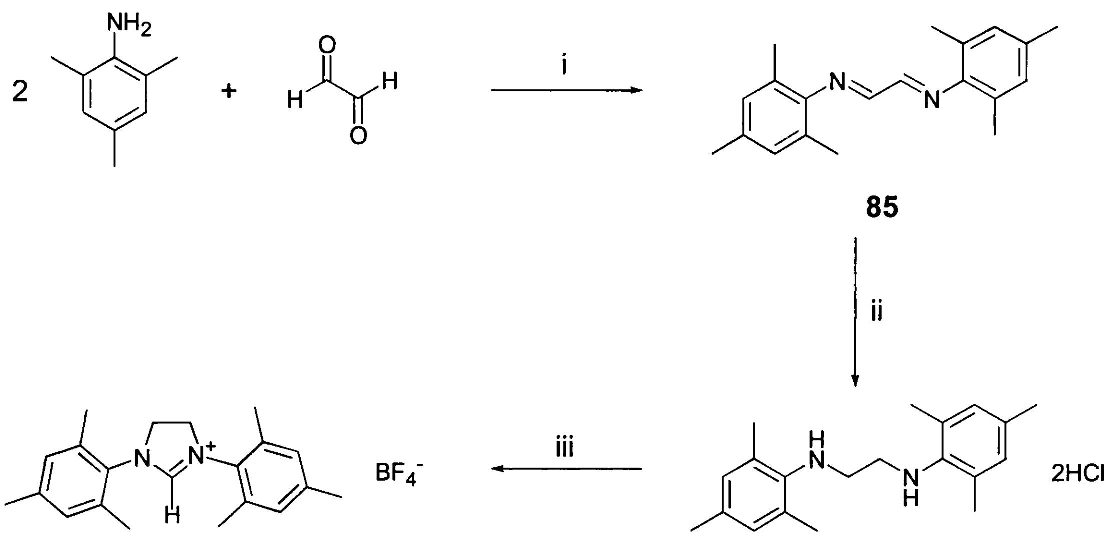
The superiority of the homogenous second-generation initiators (**19** and **20**, Figure 1-9, page 35) over their predecessor (**18**, Figure 1-7, page 32) was also reflected in an analogous improvement of the fore-mentioned polymer-supported initiators bearing

NHC ligands. As discussed in Chapter 1, the major advantages of the second-generation initiators are their increased thermal stability, coupled with improved catalytic activity that is comparable to that of the early transition metal complexes (such as **16**, Figure 1-7). Hence, we sought replacement of the PCy_3 group on our polymer-supported initiator **78-P** (Scheme 3-1) with SIMes ligand in order to test its reactivity and/or longevity.

To this end, it was necessary to make Grubbs' second-generation catalyst **20** (Figure 1-9, page 35). A precursor to Arduengo carbene ligand 1,3-dimesitylimidazolidinium (SIMes) was made in three steps following a published method.[101] First, 2,4,6-trimethylaniline and glyoxal in propanol were heated under reflux for 18 h and the reaction mixture cooled, precipitated by addition of water and the collected precipitate washed and dried to afford *dimesityl glyoxalimine* **85** (Scheme 3-14) in 90 % yield as bright yellow crystals. This imine (**85**) was then reduced with sodium borohydride and precipitated by addition of aqueous hydrochloric acid to afford *N,N'-dimesityl-1.2-diaminoethane* **86** as the hydrochloride salt in 84 % yield. Finally, diamine **86** was freed by treatment with base, then heated with triethyl orthoformate and ammonium tetrafluoroborate at 130°C for 3h, which after cooling, concentration and recrystallisation from ethanol gave 1,3-dimesitylimidazolidinium tetrafluoroborate **87** (SIMes HBF_4) in 58 % as white needles (Scheme 3-14). A methine structure (N-*CH*=N^+), showed a characteristic singlet at 8.96 ppm in ^1H NMR and a signal at 160.8 ppm in ^{13}C spectrum, which were in accordance with the published data.[67,102]

In the first instance, some problems were encountered in attempts to synthesize **20** (Scheme 3-15), following the method of Grubbs[57] or Hoveyda.[67] Both use potassium *tert*-butoxide in tetrahydrofuran to deprotonate SIMes tetrafluoroborate **87** and the resulting solution of SIMes carbene must then be *immediately* transferred *via* cannula to a solution of Grubbs' catalyst **18** in benzene. Prolonged stirring of the salt **87** with *tert*-

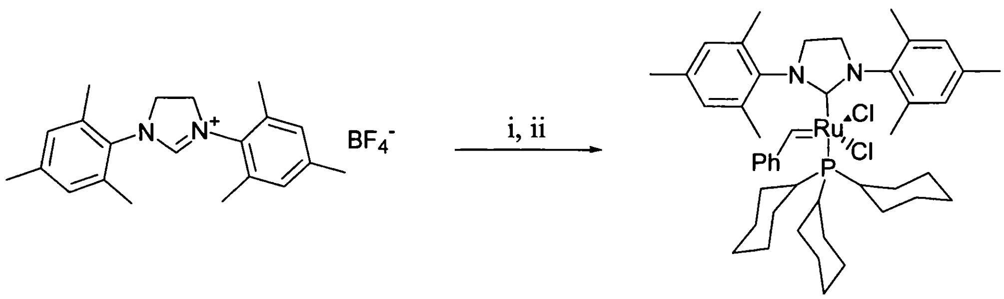
butoxide is reported to lead to incomplete conversion to the desired product.[67] The reaction was then stirred for 30 min at 80 °C, cooled and purified by column chromatography to afford **20** (Scheme 3-15) in yields ranging from 20 to 50 % after chromatography. An additional problem is that prolonged reaction times at elevated temperatures have been observed to cause the formation of the inactive four-coordinate ruthenium bis-alkoxide complex $PCy_3(OBu^t)_2Ru{=}ChPh$[103] and this may have been the cause of lower yields.



Scheme 3-14: Synthesis of SIMesH tetrafluoroborate. Reagents and conditions: i. propanol, 1h at 60°C then 18h at r.t., 90 %; ii. sodium borohydride, methanol, 0 to 20°C, 84 %; iii. triethyl orthoformate, NH_4BF_4, 130°C for 3h, 58 %.

Nolan's group[103] later published an improved one-pot synthesis of second-generation catalyst **20** using potassium *tert*-pentoxide to deprotonate SIMesH instead of *tert*-butoxide. The former is an equally strong base yet more hydrophobic, thus allowing the reaction to be carried out in hexane at room temperature. Reaction of SIMes tetrafluoroborate with potassium *tert*-pentoxide in hexane at room temperature for 1.5 h, followed by addition of Grubbs' catalyst **18** and heating at 60°C for another 2h reproducibly afforded **20** at 89 % yield as a pink-brown fine powder. ^1H NMR spectrum showed characteristic carbene (Ru=C*H*) peak shift to 19.13 ppm (20.0 ppm is characteristic for **18**) and δ_P at 29.7 ppm in ^{31}P NMR spectrum (36.6 for **18**), which was in agreement with literature.[53,57]

It is interesting that the method using tetrahydrofuran as a solvent requires immediate transfer of the generated carbene to achieve good yields, yet the hexane modification is apparently a much more stable system that can be stirred for several hours prior to addition of Grubbs' catalyst. One decomposition pathway may be due to dimerisation of the carbene and a potential solvent effect may be used as a speculative explanation here. It may be that the tetrahydrofuran efficiently solvates the 'highly active' free carbene whereas hexane encourages greater association of carbene and base so that the carbene is less reactive and thus more stable in solution.

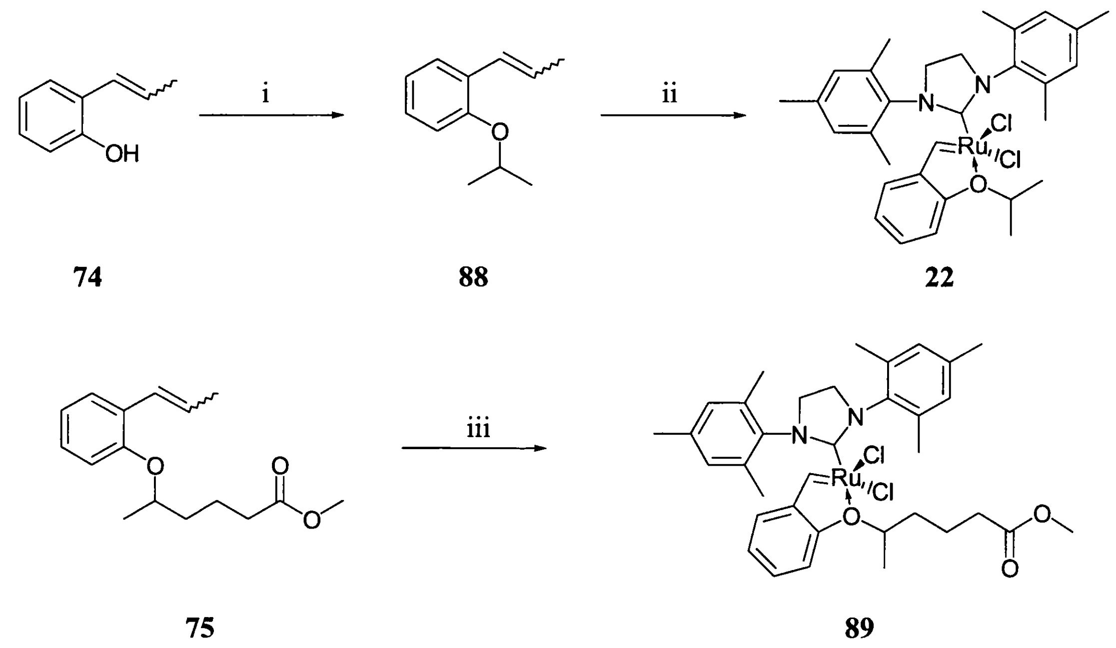


87 **20**

Scheme 3-15: Synthesis of **20**. Reagents and conditions: i. potassium *tert*-pentoxide, hexane, r.t.1.5 h; ii. Grubbs' catalyst **18** added, 60 °C for 2 h, 89 %.

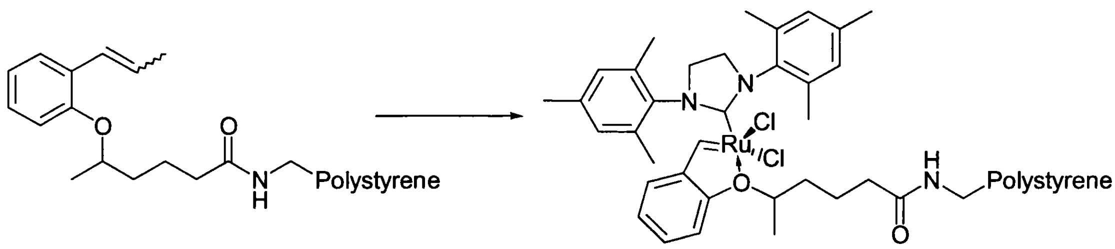
Before pursuing synthesis of polymer-supported catalyst, two homogenous catalysts (**22** and **89**) were made starting from 2-(prop-2-enyl)phenol derivatives (**74, 75**) to ensure that the reaction takes place in solution, before attempting it on a solid phase. Compound **74** was used as a starting material in synthesis of our previous polymer-supported initiator (Scheme 3-11, page 86). Hoveyda's **22** catalyst was made in two steps using the method of Garber *et al* (Scheme 3-16).[67] Alkylating 2-(prop-2-enyl)phenol **74** with 2-bromopropane in the presence of potassium carbonate and 18-

crown-6 afforded 2-(prop-2-enyl)-1-(prop-2-yloxy)benzene **88** in 84 % yield, which was then treated with Grubbs' catalyst **18** in the presence of copper(I) chloride as a phosphine scavenger to give **22** in 89 % yield. Characteristic signals in ^{1}H NMR data are observed, such as a singlet at 16.42 ppm for a carbene proton (Ru=C*H*) and a septet at 4.89 ppm (*J* 6.3) for a methane of the isopropyl group. A similar method was used to synthesise **89** from methyl 5-(2-(prop-2-enyl)phenoxy)hexanoate **75** and Grubbs' catalyst. Initiator [2-(5-methoxy-1-methyl-5-oxopentoxy)benzylidene][1,3-dimesityl-4,5-dihydroimidazol-2-ylidne]ruthenium (IV) dichloride **89** was afforded in 60 % yield. Chemical shifts in the proton NMR spectrum are similar as for the previous compound; methine proton multiplet at 4.67 ppm and carbene CH peak at 16.53 ppm.



Scheme 3-16: Synthesis of **22** and **89**. Reagents and conditions: i. 2-bromopropane, K_2CO_3, acetone, 18-crown-6, reflux, 84 %; ii. Grubbs' catalyst **20**, CuCl (1 equivalent), degassed dichloromethane, 40°C, 89 %; iii. Grubbs' catalyst **20**, degassed 1,2-dichloroethane 50 °C to r.t., 60 %.

The polystyrene-supported initiator **90-P** was made using a similar method as for our previous catalyst **78-P** (Scheme 3-11, page 86), by loading with five successive 10 mol% portions of catalyst **20** in degassed 1,2-dichloroethane to afford a dark green resin. Loading at elevated and room temperature for 2 or 4 h was compared in order to establish ideal reaction conditions. It appears that better results were obtained when loadings were carried out at elevated temperature (40°C). There seem to be no major differences in activity between the batches of resin prepared at room temperature, or at 40 °C, in the first cycle of RCM. However, the resin batches made at 40 °C perform better in recycling experiments and can be recycled up to 3 times. Batches of resin made at elevated temperature had a deep green colour whereas batches loaded at room temperature became very dark to black. The mass increase after loading five portions of **20** was greater in batches loaded at elevated temperatures (indicating higher loading of catalytic Ru species onto ligand) compared to batches made at room temperature.



77-P **90-P**

Scheme 3-17: Synthesis of second generation polystyrene-supported **90-P**. Reagents and conditions: 5 x 10 mol% **20**, degassed 1,2-dichloroethane, 40°C, 4h.

3.2.1 Testing of the Second Generation Polystyrene-Supported Initiator

Initiator **90-P** was tested for ring-closure of Cbz-diallylamine **69a** at ambient and elevated temperature. The ability of resin to be recycled was tested by using the same portion of resin and a new portion of substrate **69a**. It seems that the initial higher conversion rates (in the first run) were best achieved at room temperature, but there was no significant difference in recyclability regardless of the reactions being carried out at ambient or elevated temperature (Table 3-3).

Table 3-3: Recycling of **90-P** for the RCM of benzyl *N,N*-diallylcarbamate [a] at room and elevated temperature

Temp °C	Conversion, % [b]			
	1[c]	**2**	**3**	**4**
22	100 [d]	75	52	25
40	69 [e]	77	62	-

[a] All runs performed in non-degassed dichloromethane for 90 minutes at either room temperature or under reflux; [b] Relative integration of ^{1}H NMR; [c] cycle number, [d] reactions carried out at room temperature; [e] at 40 °C.

An attempt was also made to simplify the reaction by loading 1.2 equivalent of **20** in one portion in the presence of an equimolar amount of CuCl as a phosphine scavenger. However, this again resulted in a resin of inferior ring-closing activity compared to the product made by multiple loading of 10 mol% **20**. Despite achieving comparable ring-closing of **69a** in the first run, the initiator was not recyclable (Table 3-4). Resin made using 1.2 equivalent CuCl as a phosphine scavenger with 1.2 equivalent **20** showed poorer activity compared to the resin made using standard procedure of multiple loadings of 10 mol% **20** (Table 3-4).

Table 3-4: Testing **90-P** (made using CuCl) for RCM of benzyl *N,N*-diallylcarbamate [a] at ambient and elevated temperature

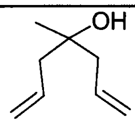
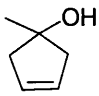
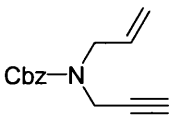
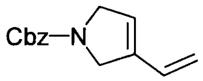
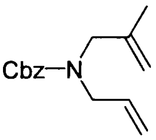
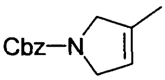
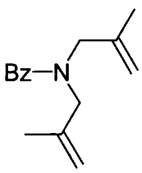
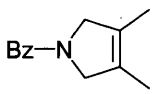
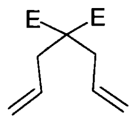
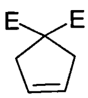
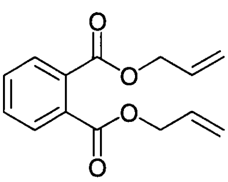
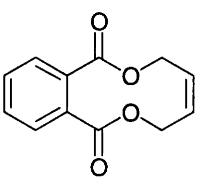
Temp °C	Conversion, % [b]	
	1[c]	**2**
22	67 [d]	2
40	80 [e]	-

[a] All runs performed in non-degassed dichloromethane for 90 minutes at either room temperature or under reflux; [b] Relative integration of ^{1}H NMR; [c] cycle number, [d] reactions carried out at room temperature; [e] at 40 °C.

Initiator **90-P** was also tested for ring-closing of several other selected substrates (Table 3-5). Ability to perform alkyne-alkene metathesis was tested on substrate **70a** (entry 2). Compounds **71a** and **72a** (entries 3 and 4) were used to explore activity in RCM of substituted terminal dienes. Mono-substituted diene **71a** (entry 3) underwent good conversion (82 %, as observed by NMR spectra), but the isolated yield was much lower

(54 %). Disubstituted diene **72a** (entry 4) however, did not react at all. It is notable that initiator **90-P** achieved better conversion rate for diethyl diallylmalonate **73a** in 2h (entry 5) than our first initiator **62-P** (43 % in 90 min). Ring-closing metathesis of diene **91a** was attempted in order to investigate if resin **90-P** could initiate ring-closure of larger rings (entry 6).

Table 3-5: Testing the ability of supported initiator **90-P** to perform ring-closing metathesis.

Entry	Substrate [a]	Product [b]	Conversion (%) [c]	Yield (%) [d]
1	 **66a**	 **66b**	63	-
2	 **70a**	 **70b**	?	-
3	 **71a**	 **71b**	82	54
4	 **72a**	 **72b**	0	0
5	 **73a**	 **73b**	85	-
6	 **91a**	 **91b**	?	-

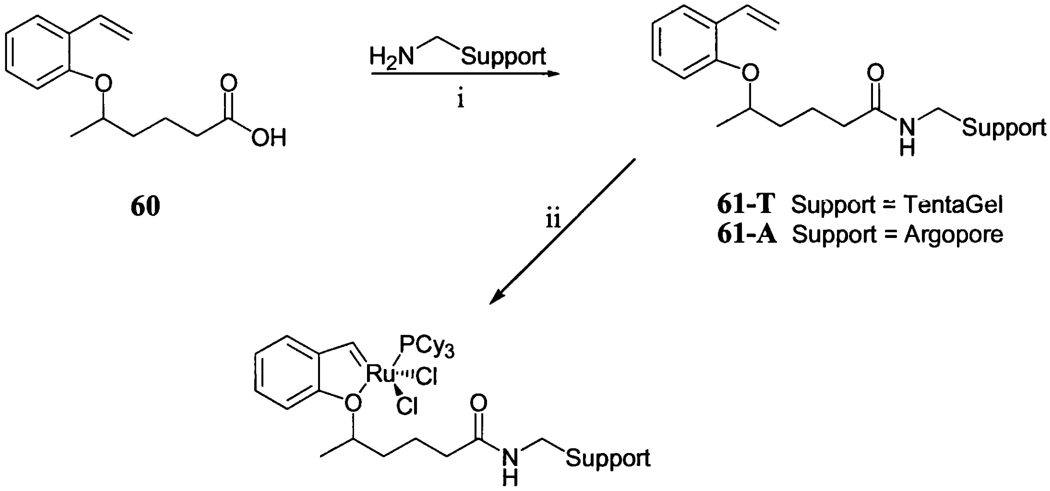
[a] All entries substrate concentrations 0.22 M (0.11 mmol substrate, 0.5 cm^3 dichloromethane), 25 mg of resin, 40°C, 2h; [c] relative integration of ^1H NMR spectra of unpurified reaction mixture; [d] isolated yields ; Cbz – benzyloxycarbonyl, Bz – benzoyl, E – CO_2Et.

Initiator **90-P** also exhibited good activity in cross-metathesis of estragole **49** and (Z)-1,4-diacetoxy-but-2-ene **50** (Scheme 3-10, page 84). The alkenes (0.1 mmol of **49** and 0.2 mmol **50**) were heated (40 °C) with initiator **90-P** (25 mg) in non-degassed dichloromethane for three hours and the products were then isolated from reaction mixture using column chromatography. Heterodimer **52** was afforded in 76 %, homodimer **51** in 14 % (based on **49**) and 61 % of scrambled (Z/E)-DAB **50** was recovered (based on **50**). This is better than what was observed with our first generation initiator **62-P** or when using homogenous Grubbs' catalyst **18**.

This polymer-supported initiator shows good overall activity for ring-closing and cross-metathesis, and is more active towards some substrates compared to its first-generation predecessor **62-P**. However, its recycling capabilities still do not match those achieved by our first generation polystyrene-supported initiator made from the 5-(2-vinylphenoxy)hexanamide-polystyrene **62-P**. We believe this is due to reaction rate differences between terminal and internal alkene functionality in the starting materials as discussed earlier (Scheme 3-13, page 87).

3.3 Polymer-Supported Initiators for Use in Protic Solvents

The ability to perform olefin metathesis in polar protic solvents is an attractive area of research in olefin-metathesis development and has so far been addressed by the development of water-soluble phosphine ligands.[74,76] The effect of different polymer supports on the solubility and catalytic activity of the complex was briefly examined at this stage. We first opted for polystyrene based macroporous resins. Although also made of polystyrene, these resins can be used in solvents in which polystyrene does not swell (e.g. methanol) as the beads of the macroporous supports contain pores sufficiently large to allow solvent to diffuse to functional groups bound to the polymer, without pre-swelling of the resin. In theory, this allows reagents/substrates to reach the catalytic sites and the reaction can take place in the pores. Amino-functionalised TentaGel (0.3 mmol/g) was coupled to **60** using the same procedure as for polystyrene bound **61-P**. TG-ligand complex **61-T** (Scheme 3-18) was treated with 1 equivalent of **18** to afford TentaGel-supported initiator **62-T**.



60

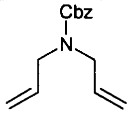
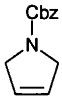
61-T Support = TentaGel
61-A Support = Argopore

62-T Support = TentaGel
62-A Support = Argopore

Scheme 3-18: Synthesis of TentaGel and Argopore-supported alkylidene ruthenium. Reagents and conditions: i. diisopropylcarbodiimide, 1-hydroxybenzotriazole, dimethylformamide : dichloromethane 1:1, r.t.; ii. Grubbs' catalyst **18** (1 x 1 equivalent or 5 x 10 mol%), degassed 1,2-dichloroethane, r.t.

TentaGel-supported catalyst **62-T** was tested for activity using ring-closing metathesis of benzyl *N,N*-diallylcarbamate in non-degassed methanol. Overnight reaction using 100 mg of TentaGel resin gave only 19% conversion (using initiator obtained by loading with equimolar **18**) of starting material. The lower conversion presumably reflects the inherent lower loading of TentaGel resin, but does suggest that it may be possible to make a suitable polymer-supported catalyst that would be active in protic solvents.

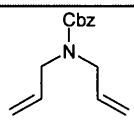
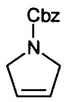
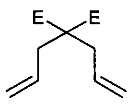
Table 3-6: Testing of **62-T** for RCM of **69a**.

Substrate	Product	Convesion
		19 %

Performed with 25 mg of substrate and 100 mg of **62-T** in methanol, at room temperature for 20 h.

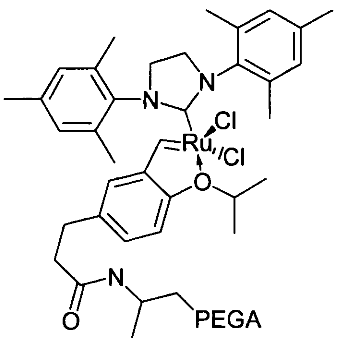
High loading macro-porous polystyrene based amino-methyl Argopore HL® resin was selected next, derivatised by reacting with **60** and ruthenium was loaded by 5 subsequent 2h reactions with 0.1 equivalent of Grubbs' catalyst (Scheme 3-18). The activity of obtained resin was comparable to the polystyrene-supported **62-P** (and **78-P**) when used in dichloromethane, but was lower when used in methanol (Table 3-7).

Table 3-7: Olefin metathesis using Argopore™ supported complex **62-A** [a]

Entry	Substrate [b]	Product	%Conversion [c] DCM	%Conversion [c] Methanol
1			77	15
2			18	N/A

[a] Performed with 25 mg of substrate and 25 mg of **62-A** in non-degassed dichloromethane or methanol, r.t., 90 min; [b] Cbz = benzyloxycarbonyl, E = CO_2Et; [c] Relative integration of ^1H NMR.

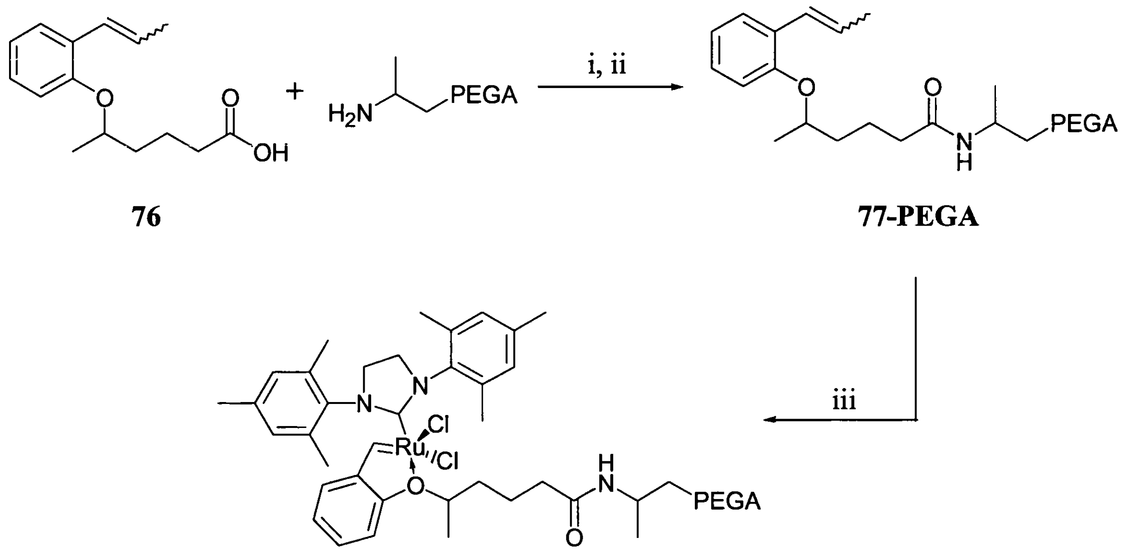
Our attempt to employ macroporous resins to support first generation initiator **62** met with limited success. Development of an Argopore-supported second-generation initiator was under consideration, when similar work was published by Blechert group using amino-PEGA as support.[98] Amino-PEGA (Novabiochem) is polyethylene glycol - acrylamide based resin that has good swelling properties in methanol, water, dichloromethane and dimethylformamide and offers a loading that is intermediate between TentaGel and polystyrene. Connon and Blechert prepared a PEGA-supported phosphine-free alkylidene ruthenium **92** (Figure 3-3) that could be used in protic solvents such as methanol and water. When used with polar substrates this catalyst showed activity in methanol comparable or better than that observed in dichloromethane, and somewhat poorer activity in H_2O (depending on the substrates used). For example RCM of *N,N*-diallylammonium chloride proceeded in 57 % yield in methanol, 33 % yield in dichloromethane and only 11 % yield in H_2O (45 °C, overnight). On the other hand, some substrates, such as hepta-1,6-diene-4-ol were converted to the cyclised product in 96 % in D_2O (room temperature, overnight).[98] This work showed that hydrophobic ruthenium alkylidene, when attached to a hydrophilic support can exhibit considerable metathesis activity in protic solvents. Because of our previous limited success with Argopore-supported **62-A**, that showed lower activity in methanol than in dichloromethane, Blechert's results encouraged us to examine PEGA as a support for our ligand.



92

Figure 3-3: Blechert's PEGA-supported second generation ruthenium alkylidene initiator.

Synthesis was similar to that of a polystyrene-supported initiator **90-P**. 5-(2-(Prop-1-enyl)phenoxy)hexanoic acid **76** was coupled to amino-PEGA (loading 0.40 mmol/g) in the presence of diisopropylcarbodiimide and hydroxybenzotriazole to give the immobilised ligand **77-PEGA** (Scheme 3-19), which was negative to Kaiser test for identification of primary amines. However, the parent amino-PEGA resin was also negative to the Kaiser test, so to ensure that all the amino groups were functionalised, the resin was subjected to acylation using a large excess of acetic anhydride. After rinsing and drying, the immobilised ligand was treated with 0.1 equivalent of catalyst **20** in 1,2-dichloroethane at 40 °C for 4h, then washed, dried and the loading procedure was repeated further four times to afford a dark green resin **90-PEGA**.

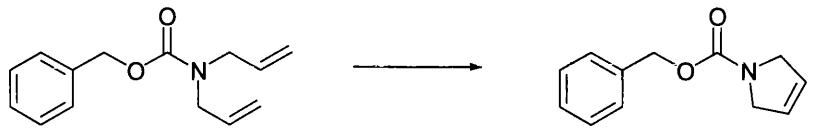


Scheme 3-19: Synthesis of PEGA-supported catalyst **90-PEGA**. Reagents and conditions: i. **76** (5 eq), diisopropylcarbodiimide (5 eq), 1-hydroxybenzotriazole (5 eq), PEGA-NH_2 (1 eq), dimethylformamide, r.t., 16 h; ii. **77-PEGA** (1 eq), Ac_2O (30 eq), triethylamine (35 eq), DMAP (3 eq), dichloromethane, r.t., 15h; iii. **77-PEGA** (1 eq), **20** (0.1 eq), degassed 1,2-dichloroethane, 40 °C, 4h, repeated 5 times.

As the parent resin was negative to Kaiser analysis and this test could not be used as a reliable proof of complete coupling of amino-PEGA to **76**, an *N-capped amino-PEGA* was made in order to examine whether the catalyst **20** was actually reacting with the ligand or being non-specifically adsorbed onto the resin surface. This resin was then loaded with 1 equivalent of Grubbs' catalyst **18** to afford a pale pink resin that was completely inactive to ring-closing metathesis of Cbz-diallylamine **69a.**

The ability of **90-PEGA** to initiate ring-closing metathesis in different solvents at ambient and elevated temperature was tested on Cbz-diallylamine **69a** using our standard method. However, we observed much lower conversion rates for ring-closure of **69a** in methanol compared to dichloromethane. We also noted that better activity was achieved at room temperature than at 40 °C, which does not compare well with the results reported by Connon and Blechert (see Table 3-8).

Table 3-8: Ring-closing activity of **90-PEGA** in different solvents at different temperatures.



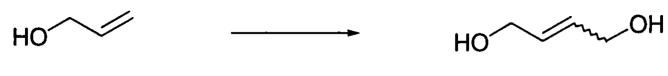
69a **69b**

Solvent [a]	Temperature (°C)	Conversion (%) [b]
Dichloromethane	room temperature	85
Dichloromethane	40	29
Methanol	room temperature	38
Methanol	40	22

All reactions carried out with 25 mg of resin **90-PEGA**, 25 mg of Cbz-diallylamine **69a** (0.11 mmol, 22 mM) in 0.5 cm^3 of solvent for 90 min; [a] all solvents are non-degassed; [b] relative integrations of ^1H NMR spectra of unpurified reaction mixtures

The ability of **90-PEGA** to perform cross-metathesis in different solvents was also tested using dimerisation of allyl alcohol. In this case, we observed similar conversion rates in dichloromethane and methanol, but no activity in D_2O (see Table 3-8). These reactions were carried out under the same conditions as reported by Blechert's group[98] for their PEGA-supported catalyst **92**. We observed good reaction rates in dichloromethane and methanol (Table 3-9), but our initiator was inactive in D_2O, whereas theirs successfully induced dimerisation of allyl alcohol to 80 % yield.

Table 3-9: Homo-coupling of allyl alcohol using **90-PEGA** in different solvents



93

Solvent [a]	Temperature (°C)	Conversion (%) [b]
Dichloromethane	40	75
Methanol	40	71
D_2O	40	0

All reactions carried out with 25 mg of resin **90-PEGA**, 6 mg of allyl alcohol (0.1 mmol, 20 mM) in 0.5 cm^3 of solvent for 12 h; [a] all solvents are non-degassed; [b] relative integrations of ^1H NMR spectra of unpurified reaction mixtures.

3.4 Summary

A polystyrene-supported ruthenium alkylidene **62-P** was designed, synthesised and its ring-closing and cross-metathesis activity tested using suitable substrates. This initiator could be used in non-degassed solvents and could be recycled up to five times with minimal loss of activity. An attempt to improve reproducibility of the synthetic route using different starting material to produce a similar polymer-supported linker resulted in an initiator with inferior activity. A second-generation phosphine-free polystyrene-supported initiator bearing N-heterocyclic carbene was synthesised (**90-P**), which exhibited increased RCM of some substrates, but showed limited recyclability due to slower rate of reassociation of the active species onto the supported ligand. In a quest to make olefin metathesis available in protic solvents, two initiators attached to macroporous polystyrene supports were prepared and tested (**62-T**, **62-A**). Their activity was lower compared to polystyrene-supported initiator **62-P**. Finally, a second-generation PEGA-supported initiator **90-PEGA** showed reasonable activity in methanol, but contrary to our expectations based on the literature, was inactive in D_2O.

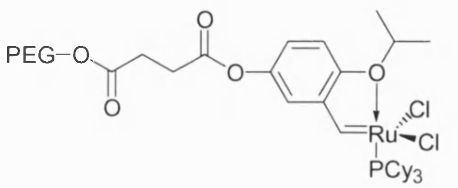
4 SUMMARY AND FUTURE PROSPECTS

The concept of dynamic combinatorial chemistry was briefly examined by exploiting a literature based competition experiment of three carbonic anhydrase inhibitors self-assembled through imine formation from a selection of building blocks (three amines and an aldehyde). This experiment showed amplification of the species with the highest affinity to the enzyme. Our further interests in the area involved employment of cross-metathesis as a tool for bond formation between potential alkene building blocks in the presence of biological targets (particularly enzymes). Initial investigations into olefin metathesis of selected substrates met with difficulties regarding activity and reproducibility of olefin scrambling reactions initiated by the commercially available initiator (Grubbs' catalyst **18**), which we sought to address by the development of novel polymer-supported initiators for olefin metathesis.

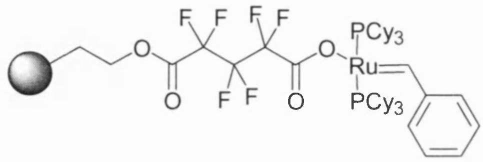
Although our investigations into DCC did not yield results of major significance they have created the need and paved the way for the development of easy to handle polymer-supported initiators for olefin metathesis that could be used for DCL generation.

A polystyrene-supported initiator **62-P** was prepared in several steps and tested for ring-closing and cross-metathesis activity of selected substrates. This initiator could be used in non-degassed solvents and recycled up to five times without addition of additives to prolong its activity as was necessary for previously reported Barrett's initiators **34** and **35**.

Since our initial communication,[104] several notable papers were published in the area of polymer-supported olefin-metathesis catalysts. Blechert's[99] Merrifield-polystyrene-supported second generation **79** (Figure 3-1) and Hoveyda's macromolecular and glass supported initiators **80** and **81** and later **82** to **84** (Figure 3-2, page 90) were already described in the previous chapter. At the same time Yao reported a soluble polymer polyethyleneglycol-bound ruthenium carbene complex **94**.[105] This initiator showed good activity for RCM of various diene substrates and remarkable recycling properties, with only slight drop in activity after 8 runs. The author contributes this to slow, competing decomposition of the propagating species, presumably a monophosphine Ru carbene ($Cy_3PCl_2Ru{=}CH_2$). Initiator **94** is bound to a soluble hydrophilic polymer which might be used in polar solvents and is easily removed from reaction mixture by precipitation with diethyl ether. Later in 2001, Nieczypor *et al.* reported polystyrene-supported initiator **95**,[106] that was active in RCM of various dienes and showed moderate recyclability.



94



95

96

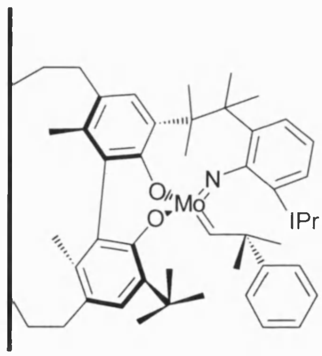


Figure 4-1: Recent developments of polymer-supported initiators for olefin metathesis.

Most recent developments in the field of polymer-supported initiators for olefin metathesis include the first polymer-supported and recyclable molybdenum based chiral initiator for enantioselective olefin metathesis (**96**) prepared recently by the groups of

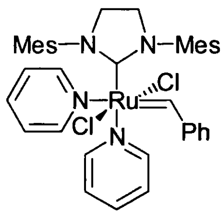
Hoveyda and Schrock.[107] In most cases, this polymer-supported catalyst gives similar levels of enantioselectivity as its homogenous equivalent, with the added advantage that it can be recycled and that Mo contamination in the unpurified product is reduced.

Further development of our initiators followed. We investigated the possibility of simplifying the synthetic route to our initiator with limited success and prepared a second-generation polystyrene-supported initiator. Attempts were made to develop first- and second-generation olefin-metathesis initiators that could be used in protic solvents, by attachment to suitable support, again with moderate success.

The work described in this thesis has laid foundations for further expansion of the olefin-metathesis initiator project within our group. A reproducible new synthetic route to 5-(2-vinylphenoxy)hexanoic acid has already been devised (Regourd) and the investigation of the effects of structure changes on activity is currently under way. Employment of our supported initiators for olefin metathesis in DCL generation, though not fully explored in this thesis, is still a subject of study by our group.

4.1 Ideas for Future Work

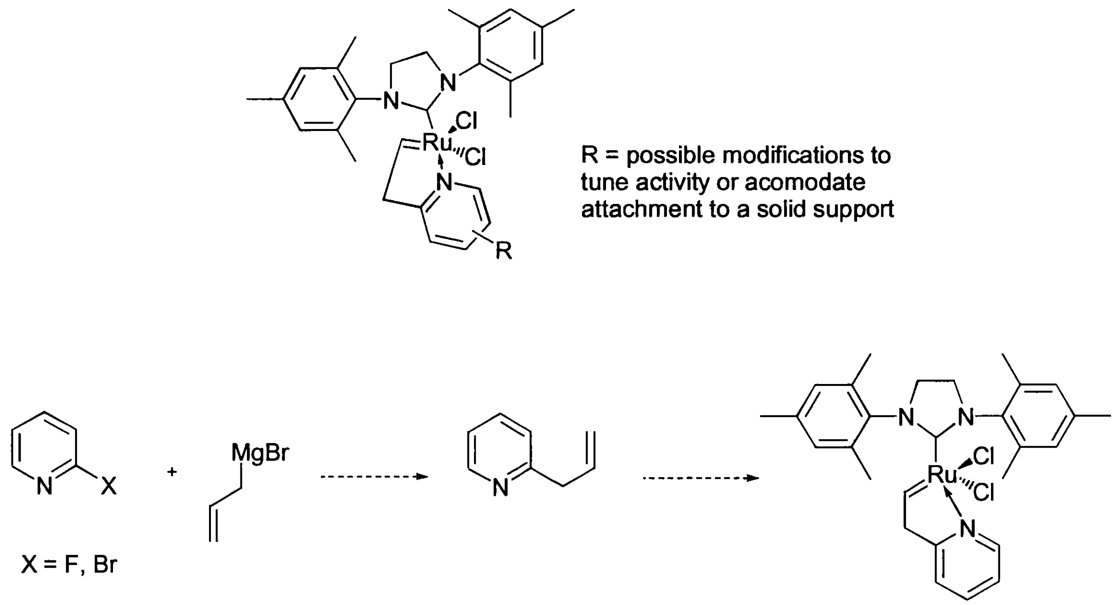
Two recent publications reported pyridine-coordinated ruthenium catalysts[108] that have some advantages. The catalyst **97** (Figure 4-2) reported by Love *et al.*[109] is the fastest ruthenium based initiator of cross-metathesis of acrylonitrile which has so far been resistant to cross-metathesis. There is evidence that this is due to the lability of the pyridine ligands which allows for the fast initiation of the reaction. It is possible that this or similar initiators may be able to induce cross-metathesis of other substrates that were previously inert to it.



97

Figure 4-2: Bis-pyridine coordinated ruthenium complex.

Replacing Hoveyda's isopropoxystyrene group with a 2-allylpyridine should produce a similar five-membered ruthenium ring structure (Figure 4-3), offering stabilisation of the ruthenium analogues to **22**. Unfortunately, there was insufficient time to develop this idea, but hopefully materialisation of this suggestion will be taken on by the rest of our group. It remains to be seen if this species would be active.



98

Figure 4-3: Ideas for pyridine chelate initiator and its synthesis.

A short and simple synthetic route to species **98** is also suggested (Figure 4-3). If the suggested species is successfully prepared and proves active, further alterations and possible attachment to solid support could be explored.

5 EXPERIMENTAL

5.1 General Experimental

Glassware was dried in a 160 °C oven and then cooled under nitrogen or argon prior to use. Tetrahydrofuran was obtained by distillation from sodium / benzophenone. All other chemicals were purchased from commercial suppliers and used as supplied. Analytical TLC was performed with silica gel 60 F254 pre-coated aluminium plates (0.25 mm thickness) from Merck, with visualisation by UV light (254 nm) or development with phosphomolybdic acid in ethanol unless otherwise stated. Flash chromatography was performed on silica gel 60 (230-400 mesh) from BDH. Infrared spectra were recorded on Perkin-Elmer 782 single wave spectrophotometer or Perkin-Elmer Spectrum RX I FT-IR System. ^{1}H and ^{13}C NMR were obtained on JEOL JMN GX-270 or Varian EX-400 NMR spectrometers; the field frequency for ^{1}H spectra was 399.8 MHz (Varian) or 270.0 MHz (JEOL), for ^{13}C 100.5 MHz (Varian) and 67.9 MHz (JEOL), for ^{31}P 109.3 MHz (JEOL). Multiplicities are given as s for singlet, d for doublet, t for triplet, q for quartet and more complex multiplicities are presented as a combination of these letters or as m for multiplet. Broad signals are presented with br. *J* values are given in Hz. Chemical shifts were measured in ppm relative to internal tetramethylsilane (peak at 0 ppm) or residual solvent peak. Mass spectra were recorded at University of Bath Mass Spectrometry Service on a VG Analytical Autospec Mass Spectrometer using +ve and –ve fast atom bombardment (FAB) with 3-nitrobenzyl alcohol as a matrix. Melting points were determined using a Reichtert-Jung Therm Galen Kofler block and are uncorrected. RP-HPLC analysis was performed on Varian Dynamax model SD-200 using a reverse phase column (Kingsorb C18, 5µm, 150 x 4.60 mm, Phenomenex). Degassing was achieved by three cycles of freeze-pump-thaw.

UV absorbance and the activity of carbonic anhydrase

Carbonic anhydrase from bovine erythrocytes (CA) was purchased from Sigma and tested for activity prior to each use. Carbonic anhydrase (6 mg, 0.2 mmol) in phosphate buffer pH 6.0 (2 cm^3, 20 mM) was scanned for UV absorbance on a Philips UV/VIS spectrometer, wavelength range 220 to 360 nm, with buffer used as blank. Maximum absorbance observed at 272.8 nm, A 2.614.

Spectrophotometric measurement of CA activity [95]

Stock solutions: i. phosphate buffer pH 7.5, 20 mM; ii. 4-nitrophenyl acetate (p-NPA), 0.5 mM in acetonitrile (substrate); iii. 4-nitrophenol 0.5 mM in acetonitrile (product of catalysis); iv. carbonic anhydrase 0.1 mM in phosphate buffer pH 7.5; v. 4-sulfamoyl-*N*-allylbenzamide (**46**) 0.3 mM in phosphate buffer pH 7.5 as CA inhibitor.

Four spectrophotometry cuvettes were filled with different solutions and placed in numbered positions in a Perkin-Elmer Lambda 40 UV-VIS spectrometer:

1. 2 cm^3 buffer pH 7.5 (20 mM, as zero)

2. p-NPA (50 μM) in buffer: 0.3 cm^3 of ii and 2.7 cm^3 buffer

3. p-NPA (50 μM) + CA (1 μM): 2.7 cm^3 buffer, 0.3 cm^3 of ii and 30 μL of iv.

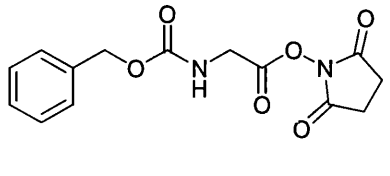
4. p-NPA (50 μM) + CA inhibitor **46** (1 μM) + CA (1 μM): 2.7 cm^3 buffer, 0.3 cm^3 ii, 10 μL of v. and 30 μL of iv.

Absorbance was automatically measured from each cuvette every minute for 1 h. Absorbance of the solution of 4-nitrophenol (50 μM) was used to estimate the maximum absorbance (if all substrate was transformed to 4-nitrophenol). Data were processed by UV KinLab software package, supplied with the spectrometer.

5.2 Dynamic Combinatorial Chemistry Experiments

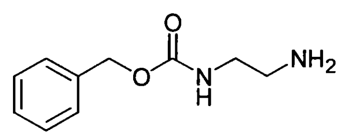
5.2.1 Synthesis of Starting Materials and HPLC Standards

N-Benzyloxycarbonylglycine 2,5-dioxopyrrolidin-1-yl ester 99[110]



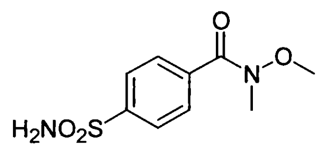
A solution of 1,3-dicyclohexylcarbodiimide (0.54 g, 2.6 mmol) in dichloromethane (1 cm^3) was added to a stirring solution of 4-(dimethylamino)pyridine (3 mg, 24 µmol), *N*-hydroxysuccinimide (0.30 g, 2.6 mmol) and triethylamine (0.27 g, 2.6 mmol) and *N*-(benzyloxycarbonyl)glycine (0.50 g, 2.4 mmol) in dichloromethane (5 cm^3) and the mixture was stirred at room temperature for 2 h. The reaction mixture was then evaporated under reduced pressure and the residue shaken with ethyl acetate and filtered through Celite 521 to remove *N,N'*-dicyclohexylurea. The filtrate was washed once with saturated brine, dried with anhydrous sodium sulfate and evaporated under low pressure to give the ester (1.00 g) as crude colourless gum with white precipitate; δ_H[110] (400 MHz, $CDCl_3$) 2.82 (4H, s, COC_2H_4CO), 4.33 (2H, d, *J* 5.9, NHCH_2CO), 5.14 (2H, s, CH_2Ph), 5.49 (1H, br s, NH), 7.34 (5H, m, Ar).

Benzyl N-(2-aminoethyl)carbamate 38[90,111]



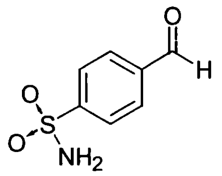
A solution of methanesulfonic acid (5.8 g, 60 mmol) in water (5 cm^3) was carefully added to a stirring solution of ethane-1,2-diamine (1.9 g, 32 mmol) in water (5 cm^3) at room temperature until pH 3.8 was achieved (pH-meter). The solution was diluted with ethanol (15 cm^3), vigorously stirred at room temperature and treated by alternate dropwise addition of solutions of benzyl chloroformate (4.9 g, 29 mmol) in 1,4-dioxane (5 cm^3) and 50% w/v aqueous sodium acetate (~ 10 cm^3) to maintain pH between 2.8 to 3.8 (pH-meter). Stirring was continued at room temperature for 1 h and the volatiles removed at low temperature under vacuum. The residue was shaken with water and filtered to remove the dicarbamate. The filtrate was washed with toluene (3 times), treated with excess 40% aqueous sodium hydroxide solution (pH ~ 11) and extracted with toluene (3 times). The toluene layer was washed once with brine, dried with sodium sulfate, filtered and evaporated under vacuum to give a viscous, pale yellow oil which solidified after several days to give the *monoprotected ethylenediamine* **38** (1.3 g, 23%) as an amorphous white solid; mp 89-91°C; υ_{max} cm^{-1} (KBr) 3300, 3040-3020, 1685, 1560, 1535, 1480, 1260-1220; δ_H[111] (270 MHz, CD_3OD) 2.65 (2H, t, *J* 6.2, CH_2NH_2), 3.12 (2H, t, *J* 6.2 CH_2NH), 5.00 (2H, s, CH_2O), 7.26 (5H, m, ArH); δ_C (100 MHz, $(CD_3)_2SO$) 41.2 (CH_2), 43.3 (CH_2), 65.2 (CH_2), 127.5 (CH), 127.6 (CH), 128.2 (CH) 137.0 (C), 156.0 (C); *m/z* (FAB) 195 (M+H, 100%), 91 (42).

_N_-Methoxy-_N_-methyl-4-sulfamoylbenzamide 45 [92]



Method of Tanaka *et al.*:[92] 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDC) (2.39 g, 12 mmol) was added to 4-sulfamoylbenzoic acid (2.01 g, 10 mmol) and 1-hydroxybenzotriazole (1.62g, 12 mmol) in dimethylformamide (20 cm^3) and stirred for 20 min. *N,O*-Dimethylhydroxylamine hydrochloride (1.17 g, 12 mmol) and *N,N*-diisopropylethylamine (DIPEA) (2.1 cm^3, 12 mmol) were then added and the mixture stirred for 4 h at room temperature. The reaction mixture was evaporated and the residue partitioned between ethyl acetate and water. The aqueous phase was then extracted with ethyl acetate (3 times) and the combined organics then washed with saturated aqueous solution of $NaHCO_3$, brine, dried with anhydrous Na_2SO_4, filtered and evaporated to leave a white solid, which was then precipitated from ethyl acetate with hexane to give the *Weinreb amide* **45** (1.25 g, 51 %) as a white crystalline powder; mp 140-142°C (no lit. mp data); ν_{max} cm^{-1}(KBr) 3294, 3198, 1602, 1563, 1504, 1347, 1284, 1163; δ_H[92] (400 MHz, $(CD_3)_2SO$) 3.29 (3H, s, CH_3), 3.56 (3H, s, CH_3), 7.52 (2H, br s, NH_2), 7.77 (2H, d, J 8.0, ArH), 7.90 (2H, d, J 8.0, ArH); δ_C (100 MHz, $(CD_3)_2SO$) 33.8 (CH_3), 61.4 (CH_3), 125.7 (CH), 128.4 (CH), 137.9 (C), 145.6 (C), 168.2 (C); *m/z* (FAB) 268 (M+Na, 75%), 245 (M+H, 100), 184 (18).

4-sulfamoylbenzaldehyde 39

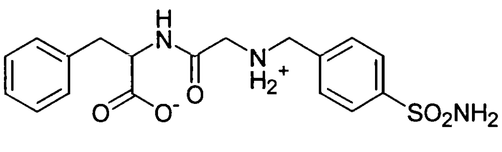


Method 1: Raney nickel, 50% in water (a small amount) was added to a solution of 4-cyanobenzenesulfonamide (250 mg, 1.4 mmol) in formic acid (5 cm^3) and the mixture heated under reflux for 30 min. The reaction mixture was cooled, then filtered through wet Celite 521. The filtrate was then extracted with ethyl acetate (3 times), washed with brine, dried with anhydrous Na_2SO_4, filtered and evaporated to leave a viscous yellow oil. The oil was dissolved in ethyl acetate and precipitated with hexane to give *4-sulfamoylbenzaldehyde* **39** (130 mg, 51%) as pale yellow powder.

Method 2 (adapted method from Tanaka *et al.*):[92] Lithium aluminium hydride (140 mg, 3.56 mmol) was added cautiously, to a stirring solution of *N*-methoxy-*N*-methyl-4-sulfamoylbenzamide (700 mg, 2.87 mmol) in dry tetrahydrofuran (10 cm^3) under nitrogen at 0°C. The reaction mixture was stirred at 0°C for 10 min, then heated to 50°C. After 6 h the reaction was cooled to 0°C, quenched by adding aqueous HCl (1 M) and the resulting mixture extracted with ethyl acetate (3 times). The combined organics were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to leave a slightly yellow gum, which was then purified by column chromatography on silica gel using 1:1 ethyl acetate : hexane as eluent to afford a white amorphous solid, which was precipitated from ethyl acetate with hexane to give *4-sulfamoylbenzaldehyde* **39** (300 mg, 56 %) as a white powder; mp 116-118°C (lit.[91] mp 117-118°C); ν_{max} cm^{-1} (KBr) 3320, 3240, 1705, 1685, 1590, 1340, 1160; δ_H[92] (270 MHz, $(CD_3)_2SO$) 7.61 (2H, br s,

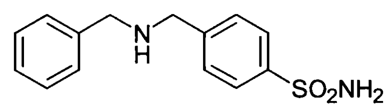
NH_2), 8.02 (2H, d, *J* 8.1, ArH), 8.10 (2H, d, *J* 8.1, ArH), 10.09 (1H, s, CHO); δ_C (68 MHz, $(CD_3)_2SO$) 126.5 (CH), 130.2 (CH), 138.2 (C), 148.8 (C), 192.7 (C); *m/z* (FAB^-) 337 (M-H+3-NBA, 45 %), 184 (M-H, 100).

N-(N-(4-Sulfamoylbenzyl)glycyl)-L-phenylalanine 40b



Sodium triacetoxyborohydride (280 mg, 1.40 mmol) was added to a stirring mixture of glycyl-L-phenylalanine (60 mg, 0.27 mmol), 4-sulfamoylbenzaldehyde **39** (56 mg, 0.30 mmol) and acetic acid (20 μL, 0.35 mmol) in acetonitrile (4 cm^3) and the mixture stirred at room temperature for 48 h. The reaction mixture was then evaporated, dissolved in a small volume of water, loaded onto 5g Isolute® C18 cartridge (Jones Chromatography) and eluted with water. The aqueous fractions were collected and freeze-dried to afford *N-(N-(4-sulfamoylbenzyl)glycyl)-L-phenylalanine* **40b** (60 mg, 51 %) as a white powder; mp 158–160 °C; ν_{max} cm^{-1} (KBr) 3470, 3246br, 1686, 1639, 1557, 1330, 1280, 1154; δ_H (400 MHz, D_2O) 2.76 (1H, dd, *J* 13.9, 9.0, PhCH_AH_B), 3.04 (1H, dd, *J* 13.9, 4.9, PhCH$_AH_B$), 3.48 (1H, d, *J* 16.0, CH_AH_B), 3.60 (1H, d, *J* 16.0, CH$_AH_B$), 4.31 (1H, dd, *J* 9.0, 4.9, $CHCOO^-$), 4.55 (2H, s, $CH_2NH_2^+$), 7.08-7.14 (5H, m, ArH), 7.39 (2H, d, *J* 8.6, ArH), 7.72 (2H, d, *J* 8.6, ArH), δ_C (100 MHz, D_2O) 37.7 (CH_2), 40.5 (CH_2), 56.8 (CH), 63.1 (CH_2), 126.2 (CH), 127.0 (CH), 127.9 (CH), 128.8 (CH),129.3 (CH), 130.2 (C), 137.8 (C); *m/z* (ESI^-) 390 (M-H, 100 %).

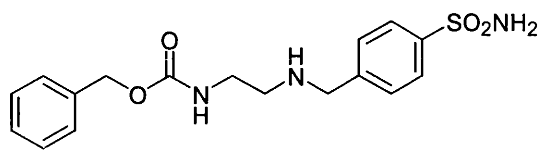
4-(Benzylaminomethyl)benzenesulfonamide 41b



Method 1: Sodium cyanoborohydride (120 mg, 1.9 mmol) was added to a stirred solution of benzylamine (0.07 cm^3, 0.6 mmol) and 4-sulfamoylbenzaldehyde **39** (100 mg, 0.5 mmol) in methanol (3 cm^3) at 0°C. The reaction mixture was stirred for 40 h at room temperature, then evaporated to dryness, shaken with ethyl acetate and extracted with HCl (1 M, 3 x 10 cm^3). The aqueous acidic phases were combined, treated with aq. NaOH (1 M) until pH 11 and extracted with dichloromethane (3 times). The combined organic layers were dried over Na_2SO_4, filtered and evaporated to leave a white gum which was recrystallised from ethyl acetate to give **41b** (28 mg, 19%) as a white solid.

Method 2 (modified method by Abdel-Magid *et al.*):[94] Sodium triacetoxyborohydride (85 mg, 0.40 mmol) was added to a suspension of 4-sulfamoylbenzaldehyde (50 mg, 0.27 mmol) and benzylamine (32 mg, 0.30 mmol) in tetrahydrofuran (5 cm^3) and the mixture stirred for 48 h at room temperature. The reaction mixture was then evaporated, partitioned between saturated aqueous solution of $NaHCO_3$ (10 cm^3) and ethyl acetate (10 cm^3) and the aqueous phase further extracted with ethyl acetate (3 times). The organic phases were combined, washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to give a white solid, which was then recrystallised from ethanol to afford *4-(benzylaminomethyl)benzenesulfonamide* **41b** (62 mg, 83 %) as white crystals; mp 141-143 °C; ν_{max} cm^{-1} (KBr) 3296, 2638, 1600, 1580, 1498, 1409, 1320, 1161; δ_H (270 MHz, CD_3OD) 3.63 (2H, s, CH_2NH), 3.71 (2H, s, CH_2NH), 7.14-7.23 (5H, m, ArH), 7.41 (2H, d, *J* 8.4, ArH), 7.76 (2H, d, *J* 8.4, ArH), δ_C (100 MHz, CD_3OD) 52.9 (CH_2), 53.6 (CH_2), 127.1 (CH), 128.1 (CH), 129.3 (CH), 129.4 (CH), 129.8 (CH), 140.3 (C), 143.5 (C), 145.2 (C); *m/z* (FAB) 277.1015 (M+H, for $C_{14}H_{17}N_2O_2S$ required 277.1011), 91 (80 %).

Benzyl *N*-(2-(4-sulfamoylbenzylamino)ethyl)carbamate 42b



Method 1: Sodium cyanoborohydride (95 mg, 1.5 mmol) was added to a stirring solution of Benzyl *N*-(aminoethyl)carbamate **38** (194 mg, 1.0 mmol) and 4-formylbenzenesulfonamide **39** (100 mg, 0.5 mmol) in methanol (3 cm^3) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 24 h, then evaporated to dryness, shaken with ethyl acetate (~ 10 cm^3) and extracted with 1 M aqueous HCl (3 times). The aqueous acidic phases were combined and aqueous NaOH (1 M) was added until pH 11 and extracted with dichloromethane (3 times). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under vacuum to leave a white precipitate which was purified by column chromatography on silica gel eluting with gradient mixtures of methanol : dichloromethane : triethylamine, starting from 1:97:2 and finishing with 10:88:2, to give **42b** (27 mg, 14%) as a pale yellow solid.

Method 2 (modified method by Abdel-Magid *et al.*):[94] Sodium triacetoxyborohydride (270 mg, 1.30 mmol) was added to a suspension of 4-formylbenzenesulfonamide **39** (130 mg, 0.70 mmol), *N*-(benzyloxycarbonyl)-ethylenediamine HCl (178 mg, 0.77 mmol) and triethylamine (160 μL, 1.15 mmol) in tetrahydrofuran (10 cm^3) and the mixture stirred for 48 h at room temperature. The reaction mixture was then evaporated, partitioned between saturated aqueous solution of $NaHCO_3$ (20 cm^3) and ethyl acetate (20 cm^3) and the aqueous phase further extracted with ethyl acetate (3 times). The organic phases were combined, washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to give a white solid, which was then purified by column chromatography eluting with gradient mixtures of methanol : dichloromethane : triethylamine starting with 0.0 : 99.8 : 0.2 and finishing with 4.0 : 95.8 : 0.2. The material isolated by chromatography was then recrystallised from ethanol to afford *benzyl N-(2-(4-sulfamoylbenzylamino)ethyl)carbamate* **42b** (95 mg, 37 %) as white needles; mp 125–127°C; ν_{max} cm^{-1}(KBr) 3369, 3306, 1694, 1598, 1538, 1356, 1326, 1253, 1155; δ_H (270 MHz, CD_3OD) 2.67 (2H, t, *J* 6.2, C*H*$_2$NHCH$_2$), 3.22 (2H, m, C*H*$_2$NHCO), 3.79 (2H, s, NHC*H*$_2$Ar), 4.98 (2H, s, ArC*H*$_2$O), 7.25 (5H, m, ArH), 7.43 (2H, d, *J* 8.2, ArH), 7.78 (2H, d, *J* 8.2 ArH), δ_C (68 MHz, CD_3OD) 39.8 (CH_2), 48.1 (CH_2), 52.1 (CH_2), 66.2 (CH_2), 126.0 (CH), 127.5 (CH), 127.7 (CH), 128.2 (CH), 128.6 (CH), 137.0 (C), 142.5 (C), 144.0 (C), 157.7 (C); *m/z* (FAB) 364.1344 (M+H, for $C_{17}H_{22}N_3O_4S$ required 364.1331), 195 (20 %), 91 (54).

5.2.2 The Competition Experiment

Calibration Curves

Stock solutions (10mM) of **36**, **37**, **38**, **39**, **40b**, **41b**, and **42b** were prepared in 20mM phosphate buffer pH 6.0. Six serial dilutions of stock solution of each compound were made and each sample analysed on a Varian HPLC system at 230 nm, using C18 column (Kingsorb 5µm, 150 x 4.60 mm, Phenomenex) and eluted using a binary gradient of acetonitrile and 50mM phosphate buffer pH 6.0, starting at 10% acetonitrile in buffer and increasing to 30% acetonitrile over 30 minutes. Using area peak and concentration data for each compound calibration curves were constructed in Microsoft Excel and linear equations calculated for each compound.

The experiment

Carbonic anhydrase stock solution (0.79 mM) was made; the concentration of the solution was estimated spectrophotometrically at 280 nm, ε^{21} = 57000 M^{-1}cm^{-1} to be

0.79 mM. Enzyme activity assay of this solution was performed prior to experiment using kinetic spectrophotometric assay based on the hydrolysis of 4-nitrophenyl acetate and continuos measurement of the absorbance of the created 4-nitrophenol at 400 nm over 1h (see general experimental for full procedure).

The competition experiment with carbonic anhydrase: 0.40 cm^3 of each of the amine stock solution and 0.08 cm^3 of aldehyde **39** stock solution were mixed together with 0.68 cm^3 of enzyme stock solution and 0.04 cm^3 of 100 mM stock solution of sodium cyanoborohydride in phosphate buffer (20 mM, pH 6).

Control: 0.40 cm^3 of each of the amine stock solution and 0.08 cm^3 of aldehyde stock. were mixed together with 0.04 cm^3 of 100 mM stock solution of sodium cyanoborohydride in phosphate buffer (20 mM, pH 6) and filled up with buffer to 2.00 cm^3.

Both control and the experiment were incubated in a water bath at 25 °C for 14 days. During the incubation samples from control were occasionally taken for HPLC analysis to monitor the time needed to achieve equilibrium. After 14 days the enzyme was denatured by incubating the reaction mixture in a water bath at 80°C for 2 minutes, followed by a 30 min microcentrifugation at 13000 rpm and filtration of the supernatant through a syringe filter (pore size 0.2 μm). Both experiment and control were fully analysed by HPLC after 14 days incubation (using the same conditions as for calibration curves, Table 5-1).

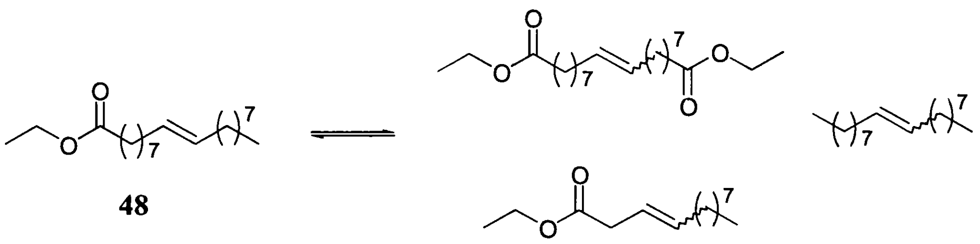
Calculated relative ratios: $(\mathbf{41b/42b})_{rel}$ = 17.5 / 0.92 = 19. Normalized value x = 17.5 / 0.92 = 19 means that the ratio of two products $(\mathbf{41b/42b})_{rel.}$ is 19 times higher with CA than without CA (Lit. [21] $x = 21$).

Table 5-1: Concentration of the aldehyde **39** and the reductive amination products (**40a, 41b, 42b**) in the presence of the enzyme and control.

Compound	Control (concentration, mM)	Experiment (concentration, mM)
39	0.006	0.007
40b	0.34	-
41b	0.055	0.07
42b	0.06	0.004

5.2.3 Olefin Metathesis in DCC

Cross-metathesis of ethyl oleate

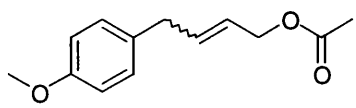


Freshly distilled ethyl oleate and 1,2-dichloroethane were degassed using the freeze-pump-thaw procedure. Grubbs' catalyst (18 mg, 0.03 mmol) was dissolved in degassed 1,2-dichloroethane (5 cm^3) and ethyl oleate **48** (1 cm^3, 2.80 mmol) was added neat in dropwise additions to the stirring mixture and left to stir under nitrogen for 60 h at room temperature. In the beginning, the colour was dark purple and during first 30 minutes it changed to brown. The sample was taken from the reaction, diluted with dichloromethane to 1mM and analysed by GCMS (Chromatogram in Figure 2-4, page 67).

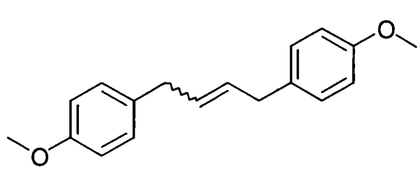
Cross-olefin metathesis of 49 and using different substrate concentrations - general procedure

Cis-1,4-diacetoxybut-2-ene **50** (116 mg, 0.68 mmol), 4-allylanisole (estragole) (50 mg, 0.34 mmol) and Grubbs' catalyst **18** (56 mg, 0.07 mmol) were dissolved in normal laboratory grade dichloromethane (1 cm^3) in a V bottomed quickfit test tube. The tube was equipped with a condenser and the reaction stirred at reflux. The reaction mixture was then evaporated, loaded on a silica gel column and eluted using a gradient of diethyl ether : hexane, starting with 3:97 and finishing with 1:9. Isolated products:

Heterodimer – **cis/trans 4-(4-methoxyphenyl)but-2-enyl acetate 52** (58 mg, 76 % based on **49**); *cis* : *trans* isomer 15 : 85 (^{1}H NMR integration); ν_{max} cm^{-1} (film) 3476, 3005, 1739, 1602, 1512; *cis* isomer δ_H[112] (400 MHz, $CDCl_3$) 2.08 (3H, s, CH_3CO), 3.41 (2H, d, *J* 7.4, CH_2Ph), 3.79 (3H, s, CH_3O), 4.73 (2H, d, *J* 7.0, CH_2O), 5.55-5.68 (1H, m, C*H*=CH), 5.76-5.82 (1H, m, CH=C*H*), 6.84 (2H, d, *J* 8.8, ArH), 7.09 (2H, d, *J* 8.8, ArH), *trans* isomer δ_H (400 MHz, $CDCl_3$) 2.06 (3H, s, CH_3CO), 3.34 (2H, d, *J* 6.7, CH_2Ph), 3.79 (3H, s, CH_3O), 4.54 (2H, dd, *J* 6.2, 1.2, CH_2O), 5.55-5.68 (1H, m, =C*H*CH_2O), 5.90 (1H, dtt, *J* 15.2, 6.7, 1.2, =C*H*CH_2Ph), 6.84 (2H, d, *J* 8.8, ArH), 7.09 (2H, d, *J* 8.8, ArH); δ_C (100 MHz, $CDCl_3$) 21.5 (CH_3), 38.1 (CH_2), 55.6 (CH_3), 65.3 (CH_2), 114.1 (CH), 125.1 (CH), 129.7 (CH), 131.7 (C), 135.2 (CH), 158.2 (C),171.0 (C); *m/z* (FAB) 297 (35 %), 220 (30), 161 (M-$CH_3CO(OH_2)^+$, 100);

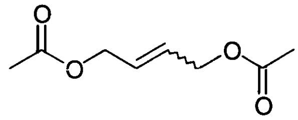


Homodimer – **cis/trans1,4-bis(4-methoxyphenyl)but-2-ene** (8 mg, 18 % based on **49**); isomer A 20 %: δ_H (400MHz, $CDCl_3$) 3.38 (4H, d, *J* 5.7, 2xCH_2), 3.72 (6H, s, 2xCH_3), 5.57-5.61 (2H, m, CH=CH), 6.74-6.80 (4H, m, ArH), 7.00-7.08 (4H, m, ArH), isomer B 80 %: δ_H (400MHz, $CDCl_3$) 3.23 (4H, d, *J* 5.2, 2xCH_2), 3.72



(6H, s, 2xCH_3), 5.52-5.56 (2H, m, CH=CH), 6.74-6.80 (4H, m, ArH), 7.00-7.08 (4H, m, ArH); *m/z* (FAB) 315 (30 %), 269.1508 (M+H, for $C_{18}H_{20}O_2$ required 269.1541), 161 (73 %), 147 (61), 73 (53);

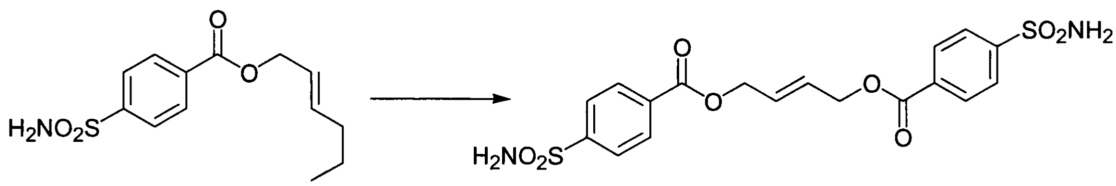
and **cis/trans 1,4-diacetoxybut-2-ene** (Z/E)-**50** (42 mg, 35 % based on **50**,



approximately *cis* / *trans* 20 : 80 %); *cis* isomer δ_H (400 MHz, $CDCl_3$) 2.04 (6H, s, 2xCH_3), 4.65 (4H, d, *J* 5.1, 2xCH_2), 5.70-5.75 (2H, m, CH=CH), *trans* isomer δ_H (400 MHz, $CDCl_3$) 2.06 (6H, s, 2xCH_3), 4.55 (4H, d, *J* 4.3, 2xCH_2), 5.81-5.86 (2H, m, CH=CH).

The reaction was repeated at several different concentrations. The concentrations, conditions and isolated yields are listed in Table 2-5, page 70.

Olefin metathesis of hex-2-enyl 4-sulfamoylbenzoate 53



53 **54**

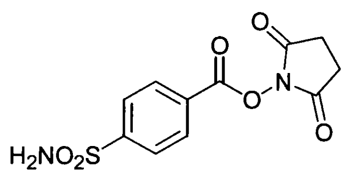
Second generation Grubbs' catalyst **20** (10 mg, 0.01 mmol) was added to a solution of hex-2-enyl 4-sulfamoylbenzoate **53** (56 mg, 0.10 mmol) in dichloromethane (2 cm^3) and the reaction mixture heated to reflux (40 °C) under nitrogen for 2h. A white precipitate (product) formed after ten minutes. The reaction mixture was then filtered and the insoluble precipitate washed with dichloromethane and methanol and dried in vacuum to afford the self-metathesis product of hex-2-enyl 4-sulfamoylbenzoate (33 mg, 74 %) as an off white powder; mp 225–227 °C; ν_{max} cm^{-1} (KBr) 3333, 3262, 1720, 1590, 1580, 1401, 1345, 1275, 1164, 1129; δ_H (400 MHz, $(CD_3)_2SO$) 4.87-4.93 (4H, m, CH_2O), 6.11-6.15 (2H, m, CH=CH), 7.59 (4H, s, NH_2), 7.96 (4H, d, *J* 8.6, ArH), 8.16 (4H, d, *J*

8.6, ArH), δ_C (100 MHz, $(CD_3)_2SO$) 65.4 (CH_2), 126.8 (CH), 128.6 (CH), 130.6 (CH), 132.9 (C), 148.7 (C), 165.0 (C); *m/z* (FAB^-) 453.0410 (M-H, for $C_{18}H_{17}N_2O_8S_2$ required 453.0415).

5.3 Polymer-Supported Alkylidene Ruthenium For Olefin Metathesis

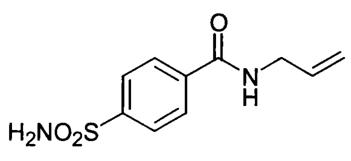
5.3.1 Synthesis of Substrates

2,5-dioxopyrolidin-1-yl 4-sulfamoylbenzoate 63[113]



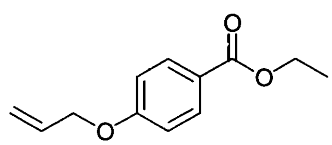
Dicyclohexylcarbodiimide (2.46 g, 11.9 mmol) in tetrahydrofuran (4 cm^3) was added to a mixture of 4-sulfamoylbenzoic acid (2.00g, 9.9 mmol), 4-dimethylaminopyridine (12 mg, 0.1 mmol) and *N*-hydroxysuccinimide (3.43g, 30.0 mmol) in dimethylformamide (15 cm^3), and the mixture stirred for 18 h at room temperature. The reaction mixture was then evaporated, shaken with ethyl acetate and filtered through Celite 521 to remove dicyclohexylurea. The filtrate was washed with brine (twice), dried with Na_2SO_4, filtered and evaporated to give **63** as a white precipitate (2.85 g); δ_H[113] (400 MHz, $(CD_3)_2SO$) 2.91 (4H, s, COC_2H_4CO), 7.71 (2H, br s, NH_2), 8.07 (2H, d, *J* 8.6, ArH), 8.29 (2H, d, *J* 8.6, ArH).

***N*-(Prop-2enyl)-4-sulfamoylbenzamide 46**



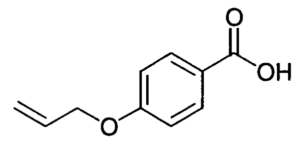
Allylamine (0.9 cm^3, 11.9 mmol) in tetrahydrofuran (1 cm^3) was added dropwise to a stirring solution of **63** (2.85 g, 9.6 mmol) in tetrahydrofuran (15.0 cm^3) at 0 °C and then stirred at room temperature for ~18 h. The reaction mixture was then acidified with 1M aqueous HCl (pH 2) and extracted with ethyl acetate (3 times). The extract was washed with brine, dried with anhydrous Na_2SO_4, filtered and evaporated to leave a white powder which was precipitated from ethanol to give **46** (1.29 g, 56%) as white amorphous solid; mp 170-175°C; ν_{max} cm^{-1} (film) 1655, 1635, 1560, 1540, 1165, 995; δ_H (400 MHz, $(CD_3)_2SO$) 3.92 (2H, tt, *J* 5.5, 1.6, C*H*$_2$NH), 5.10 (1H, dq, *J* 10.5, 1.6, CH=C*H*$_{cis}$), 5.18 (1H, dq, *J* 17.2, 1.6, CH=C*H*$_{trans}$), 5.90 (1H, ddt, *J* 17.2, 10.5, 5.5, C*H*=CH$_2$), 7.49 (2H, br s, NH$_2$), 7.89 (2H, d, *J* 8.4, ArH), 8.01 (2H, d, *J* 8.4, ArH), 8.86 (1H, t, *J* 5.5, NH); δ_C (68 MHz, $(CD_3)_2SO$) 41.9 (CH$_2$), 115.7 (CH$_2$), 126.0 (CH), 128.1 (CH), 135.2 (CH), 137.6 (C), 146.3 (C), 165.5 (C); *m/z* (FAB) 241.0647 (M+H, for $C_{10}H_{13}O_3N_2S$ required 241.0649), 173 (26 %), 97 (28).

Ethyl 4-(prop-2-enyloxy)benzoate 65[114]



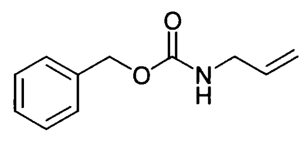
Allyl bromide (1.5 cm^3, 18 mmol) was added dropwise to a stirred solution of ethyl 4-hydroxybenzoate (1.00 g, 6 mmol) and sodium hydride (0.72 g, 18 mmol) in dimethylformamide (10.0 cm^3) at 0°C. External cooling was removed and the reaction mixture was stirred at room temperature for 60 h. The reaction mixture was then cooled to 0°C, quenched with water, extracted with diethyl ether (3 times), washed with saturated brine, dried over Na_2SO_4, filtered and evaporated to give **65** (2.04 g, 84%) as a transparent pale yellow liquid; ν_{max} cm^{-1} (film) 1710, 1600, 1580, 1510, 1310, 1270, 1100; δ_H (400 MHz, $CDCl_3$) 1.38 (3H, t, *J* 7.0, CH$_3$), 4.34 (2H, q, *J* 7.0, C*H*$_2$CH$_3$), 4.58 (2H, dt, *J* 5.5, 1.6, C*H*$_2$CH=CH$_2$), 5.31 (1H, dq, *J* 10.5, 1.6 CH=C*H*$_{cis}$), 5.42 (1H, dq, *J* 17.2, 1.6, CH=C*H*$_{trans}$), 6.05 (1H, ddt, *J* 17.2, 10.5, 5.5, C*H*=CH$_2$), 6.92 (2H, d, *J* 9.0, ArH), 7.99 (2H, d, *J* 9.0, ArH); δ_C (100 MHz, $CDCl_3$) 14.8 (CH$_3$), 60.9 (CH$_2$), 69.1 (CH$_2$), 114.4 (CH), 118.3 (CH$_2$), 123.2 (C), 131.7 (CH), 132.7 (CH), 162.3 (C), 166.4 (C); *m/z* (FAB) 207 (M+H, 39%), 181 (46), 161 (100), 111 (36), 97 (35).

4-(Prop-2-enyloxy)benzoic acid 47[115]



Aqueous potassium hydroxide (1 M, 8.58 g, 4.85 mmol) was added to ethyl 4-allyloxybenzoate **64** (0.59 g, 2.86 mmol) in 1,4-dioxane (10 cm^3) and ethanol (0.5 cm^3) and the mixture stirred at room temperature for 20 hours. The reaction mixture was then washed with ethyl acetate (3 times), acidified with aqueous HCl (1 M) and extracted with ethyl acetate (3 times). The extract was washed with brine, dried with anhydrous Na_2SO_4, filtered and evaporated under low pressure to give white crystals. The residue was then recrystallised from ethyl acetate to give *4-allyloxybenzoic acid* **47** (0.36 g, 71%) as white crystals. mp 167-169°C (lit.[115] mp 165°C from ethanol); ν_{max} cm^{-1} (KBr) 3050-2500, 1680, 1600, 1575, 1505, 1250, 1020; δ_H (400 MHz, $(CD_3)_2SO$) 4.63 (2H, d, *J* 5.3, $CH_2CH{=}CH_2$), 5.27 (1H, dd, *J* 10.5, 1.6, $CH{=}CH_{cis}$), 5.40 (1H, dd, *J* 17.2, 1.6, $CH{=}CH_{trans}$), 6.04 (1H, ddt, *J* 17.2, 10.5, 5.3, $CH{=}CH_2$), 7.00 (2H, d, *J* 9.0, ArH), 7.87 (2H, d, *J* 9.0, ArH), δ_C (100 MHz, $(CD_3)_2SO$) 69.1 (CH_2), 115.1 (CH), 118.4 (CH_2), 123.7 (C), 131.9 (CH), 133.8 (CH), 162.3 (C), 167.5 (C); *m/z* (FAB) 332 (M+H + 3-NBA, 19%), 179 (M+H, 100), 161 (26).

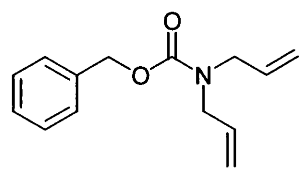
Benzyl *N*-(prop-2-enyl)carbamate 68[116]



Benzyl chloroformate (2.5 cm^3, 17 mmol) was added dropwise to a solution of allylamine (1.6 cm^3, 21 mmol) in dichloromethane (10.0 cm^3) at 0°C and the mixture stirred for 10 min, more dichloromethane (10.0 cm^3) was added and stirring continued at room temperature. After 20 h, the mixture was evaporated, shaken with diethyl ether and water, acidified with concentrated aq. HCl (until pH 1) and extracted with diethyl ether (4 times), washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to give *benzyl N-allylcarbamate* **68** (3.31 g, 99%) as a transparent pale yellow oil; υ_{max}

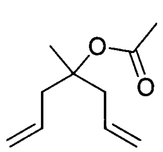
cm^{-1} (film) 3334, 1702, 1646, 1528, 1455, 1250; δ_H (400 MHz, $CDCl_3$) 3.81-3.83 (2H, m, CH_2NH), 4.88 (1H, br s, NH), 5.11-5.21 (4H, m, CH_2=CH, CH_2O), 5.83 (1H, ddt, *J* 17.2, 10.6, 5.3, *CH*=CH_2), 7.30-7.38 (5H, m, ArH), δ_C (100 MHz, $CDCl_3$) 43.9 (CH_2), 67.1 (CH_2), 116.3 (CH_2), 128.3 (CH), 128.7 (CH), 128.9 (CH), 134.6 (CH), 136.7 (C), 156.4 (C); *m/z* (FAB) 192 (M+H, 59%), 91 (100).

Benzyl *N,N*-bis-(prop-2-enyl)-carbamate 69a[117,118]



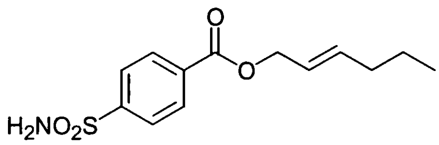
Allylbromide (2.8 cm^3, 19 mmol) was added slowly to a stirring suspension of benzyl *N*-allylcarbamate **68** (3.00 g, 16 mmol) and sodium hydride (0.36 g, 32 mmol) in dimethylformamide (20.0 cm^3) at 0°C. After addition of allylbromide the mixture was allowed to warm and the stirring continued at room temperature for 20 h. The reaction was then quenched with ice, saturated aqueous $NaHCO_3$ was added and the mixture extracted with diethyl ether (4 times), washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to give *benzyl N,N-diallylcarbamate* **69a** (3.60 g, 99%) as a transparent pale yellow liquid; ν_{max} cm^{-1} (film) 3082-3030, 1703, 1645, 1461, 1240; δ_H[118] (400 MHz, $CDCl_3$) 3.81-3.86 (4H, m, CH_2NCH_2), 5.07-5.15 (6H, m, CH_2O and 2xCH_2=CH), 5.70-5.80 (2H, br m, 2x*CH*=CH_2), 7.27-7.33 (5H, m, ArH), δ_C[118] (100 MHz, $CDCl_3$) 48.9 (CH_2), 49.5 (CH_2), 67.5 (CH_2), 116.9 (CH_2), 117.4 (CH_2), 128.0 (CH), 128.1 (CH), 128.6 (CH), 133.0 (CH), 133.7 (CH), 137.0 (C), 156.1 (C); *m/z* (FAB) 232 (M+H, 70%), 91 (100).

4-Methylhepta-1,6-diene-4-yl acetate 67a[119]



Acetic anhydride (1.0 cm^3, 9 mmol) was added to a stirring solution of 4-dimethylaminopyridine (10 mg) and 4-methylhepta-1,6-diene-4-ol (0.5 g, 4 mmol) in pyridine (5.0 cm^3) at room temperature. The reaction mixture was then heated at 60°C for 4 h, further acetic anhydride (0.3 cm^3) was added and stirring continued at room temperature for 3 days. The reaction mixture was shaken with water and extracted with diethyl ether (4 times). The combined ethereal phases were washed with water and brine, dried over Na_2SO_4, filtered and evaporated to leave a transparent yellow oil, which was purified by column chromatography on silica gel using 5:95 diethyl ether : hexane as eluent to afford *4-methylhepta-1,6-diene-4-yl acetate* **67a** (0.38 g, 56%) as a colourless liquid; ν_{max} cm^{-1} (film) 3060, 1720, 1630, 1235; δ_H[120] (400 MHz, $CDCl_3$) 1.39 (3H, s, CH_3), 1.96 (3H, s, CH_3CO), 2.49 (2H, dd, *J* 14.1, 7.4, 2xCH_AH_B), 2.63 (2H, dd, *J* 14.1, 7.4, 2xCH_AH_B), 5.06-5.12 (4H, m, CH_2=CH), 5.77 (2H, ddt, *J* 16.0, 11.3, 7.4, CH=CH_2), δ_C (100 MHz, $CDCl_3$) 22.4 (CH_3), 23.7 (CH_3), 42.6 (CH_2), 83.2 (C), 118.3 (CH_2), 132.9 (CH), 170.1 (C).

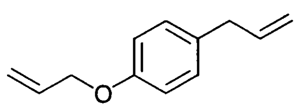
Hex-2-enyl 4-sulfonamidobenzoate 53



Dicyclohexylcarbodiimide (4.95 g, 24.0 mmol) in dimethylformamide (20 cm^3) was added to a solution of 4-sulfamoylbenzoic acid (4.00 g, 20.0 mmol), 4-dimethylaminopyridine (25 mg, 0.2 mmol) and hex-2-en-1-ol (3.00 g, 30.0 mmol) in dimethylformamide (30 cm^3) and stirred at room temperature for 20 hours. The reaction mixture was then concentrated and the residue shaken with ethyl acetate and water, filtered through Celite, and the aqueous phase extracted with ethyl acetate (twice). The combined organics were washed with water, then brine, dried with Na_2SO_4 and evaporated to leave a white powder, which was then purified by flash chromatography on silica gel using diethyl ether : hexane 1:1 as eluent. The combined fractions were evaporated and the residue precipitated from a solution in ethyl acetate with hexane to give *hex-2-enyl 4-sulfonamidobenzoate* **53** (1.62 g, 29 %) as a white powder; mp 105-106 °C; ν_{max} cm^{-1} (KBr) 3327, 3237, 1704, 1571, 1348, 1289, 1166; δ_H

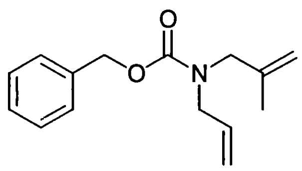
(400MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.3, CH_3), 1.44 (2H, sextet, *J* 7.3, CH_2CH_3), 2.08 (2H, q, *J* 7.3, $CH_2C_2H_5$), 4.79 (2H, dd, *J* 6.6, 1.6 OCH_2), 5.19 (2H, br s, NH_2), 5.68 (1H, dtt, *J* 15.2, 7.3, 1.6, CH=C*H*C_3H_7), 5.88 (1H, dt, *J* 15.2, 6.6, CH=C*H*CH_2O), 7.98 (2H, d, *J* 8.6, ArH), 8.16 (2H, d, *J* 8.6, ArH); δ_C (100 MHz, $CDCl_3$) 13.7 (CH_3), 22.1 (CH_2), 34.4 (CH_2), 66.5 (CH_2), 123.3 (CH), 126.3 (CH), 130.2 (CH), 134.1 (C), 137.1 (CH), 145.4 (C), 164.7 (C); *m/z* (FAB) found 284.0946 (M+H, 100 %, for $C_{13}H_{17}SO_4N$ required 284.0956), 202 (40), 184 (70).

4-(Prop-2-enyl)-1-(prop-2-enyloxy)benzene 65[121]



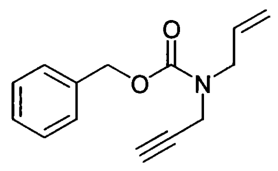
Freshly ground solid potassium carbonate (10.78 g, 78 mmol) was added to a solution of 4-allylphenol (4.26 g, 26 mmol) in wet acetone (50 cm^3, 0.5 % water) resulting in a colour change from colourless to bright yellow. This suspension was stirred for 10 min at room temperature and then neat allyl bromide (3.74 g, 31 mmol) was added dropwise from a syringe and the mixture stirred at room temperature for 24 h. More allyl bromide (1.10 g, 9 mmol) was added and stirring continued for further 72 h at room temperature resulting in discolouration of the suspension. The reaction mixture was concentrated and partitioned between water (50 cm^3) and diethyl ether (50 cm^3). The aqueous phase was extracted further with diethyl ether (3 times), and the combined ethereal phases washed with brine, dried with saturated aqueous sodium sulfate and evaporated to give a crude yellow liquid (5.05 g), which was then purified by distillation in Kugelrohr to afford *4-allyloxyallylbenzene* **65** (4.36 g, 96 %) as a colourless liquid; bp 160 °C at 2 mm Hg (Kugelrohr); ν_{max}[121] cm^{-1} (film) 3660, 3546, 1638, 1590, 1511, 1260, 1230; δ_H (270MHz, $CDCl_3$) 3.33 (2H, d, *J* 6.6, CH_2Ph), 4.59 (2H, dt, *J* 5.5, 1.5, CH_2O), 5.03-5.12 (2H, m, C*H*$_2$=CHCH_2Ph), 5.27 (1H, dq, *J* 10.3, 1.5, C*H*$_{cis}$=CHCH_2O), 5.39 (1H, dq, *J* 17.2, 1.5, C*H*$_{trans}$=CHCH_2O), 5.96 (1H, ddt, *J* 13.4, 10.3, 6.6, =C*H*CH_2Ph), 6.08 (1H, ddt, J 17.2, 10.3, 5.5, =CHCH$_2$O), 6.69 (2H, d, J 8.4, ArH), 6.81 (2H, d, J 8.4, ArH); δ_C (67 MHz, CDCl$_3$) 39.9 (CH$_2$), 70.1 (CH$_2$), 112.3 (CH), 113.6 (CH), 115.7 (CH$_2$), 117.9 (CH$_2$), 133.6 (CH), 137.7 (CH), 146.2 (C), 149.4 (C).

Benzyl *N*-(2-methylprop-2-enyl)-*N*-(prop-2-enyl)carbamate 71a



N-Allylbenzyl carbamate **68** (1.58 g, 8.2 mmol) was added dropwise *via* syringe into a suspension of sodium hydride (60 % dispersion in oil, 0.40 g, 9.9 mmol) in dimethylformamide (10 cm^3) at 0 °C and stirred for 15 min. 3-Bromo-2-methylpropene (1 cm^3, 9.9 mmol) was then added dropwise *via* syringe and the mixture warmed to room temperature and stirred for 24 h. The reaction was quenched with saturated aqueous NaHCO$_3$ (50 cm^3) and then extracted with diethyl ether (50 cm^3). The aqueous phase was extracted with diethyl ether (3 times) and the combined organics washed with brine and evaporated to give a slightly yellow oil (2.01 g), which was then purified by column chromatography on silica gel using gradient mixtures of diethyl ether : hexane 3:97 to 1:9 as eluent to afford *benzyl N-(2-methylprop-2-enyl)-N-(prop-2-enyl)carbamate* **71a** (1.76 g, 87 %) as a pale yellow liquid; ν_{max} cm^{-1}(film) 1702, 1654, 1560, 1366, 1296, 1234, 1140; δ_H (400 MHz, CDCl$_3$) 1.66-1.70 (3H, m, CH$_3$), 3.81-3.89 (4H, m, CH$_2$NCH$_2$), 4.75-4.88 (2H, m, CH_2=CCH$_3$), 5.07-5.15 (4H, m, CH$_2$O and CH_2=CH), 5.70-5.82 (1H, m, CH=CH$_2$), 7.27-7.37 (5H, m, ArH); δ_C (100 MHz, CDCl$_3$) 20.4 (CH$_3$), 48.6 (CH$_2$), 49.1 (CH$_2$), 51.8 (CH$_2$), 52.3 (CH$_2$), 67.5 (CH$_2$), 112.1 (CH$_2$), 112.4 (CH$_2$), 117.0 (CH$_2$), 117.4 (CH$_2$), 127.9 (CH), 128.1 (CH), 128.6 (CH), 133.4 (CH), 137.0 (C), 141.1 (C), 156.4 (C), (additional signals observed due to restricted rotation of the C-N bond and belong to the two rotamers); *m/z* (FAB) 246.1497 (M+H, for C$_{15}$H$_{20}$NO$_2$ required 246.1494).

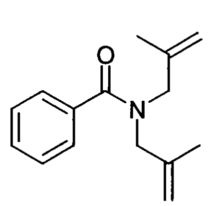
Benzyl *N*-(prop-2-enyl)-*N*-(prop-2-ynyl)carbamate 70a[122]



Benzyl *N*-allylcarbamate **68** (1.07 g, 5.6 mmol) was added dropwise *via* syringe into a suspension of sodium hydride (60 % dispersion in oil, 0.27 g, 6.7 mmol) in dimethylformamide (10 cm^3) at 0 °C and stirred for 15 min. Propargyl bromide (0.5 cm^3, 6.7 mmol) was then added dropwise from a syringe and the mixture stirred first at 0 °C for 5 min and then the ice bath removed and stirring continued at room temperature for 24 h. The reaction was quenched with ice and concentrated *in vacuo*. The residue was partitioned between water and diethyl ether and the aqueous phase extracted with diethyl ether (3 times). The combined ethereal phases were washed with brine and evaporated to give a crude brown oil, which was then purified by column chromatography on silica gel using gradient mixtures of diethyl ether : hexane 3:97 to 1:9 as eluent to afford *benzyl N-allyl-N-propargylcarbamate* **70a** (0.83 g, 65 %) as a pale yellow liquid; ν_{max} cm^{-1}(film) 3290, 2100, 1702, 1646, 1367, 1239, 1143; δ_H (270 MHz, $CDCl_3$) 2.22 (1H, t, *J* 2.4, C≡CH), 3.99-4.15 (4H, m, $CH_2CH{=}CH_2$ and $CH_2C{\equiv}CH$), 5.10-5.28 (4H, m, CH_2Ph and $CH_2{=}CH$), 5.70-5.86 (1H, m, $CH{=}CH_2$), 7.28-7.40 (5H, m, ArH); δ_C (100 MHz, $CDCl_3$) 35.8 (CH_2), 36.2 (CH_2), 48.9 (CH_2), 49.4 (CH_2), 67.9 (CH_2), 72.2 (CH), 79.4 (C), 117.8 (CH_2), 118.2 (CH_2), 128.0 (CH), 128.2 (CH), 128.7 (CH), 133.0 (CH), 136.6 (C), 139.8 (C), (additional signals observed due to restricted rotation of the C-N bond and belong to the two rotamers); *m/z* (FAB) 230.1180 (M+H, for $C_{14}H_{16}NO_2$ required 230.1181), 91 (100).

***N,N*-Bis(2-methylprop-2-enyl) benzamide 71a**

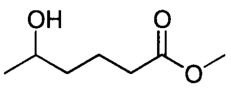
A solution of benzamide (0.55 g, 4.5 mmol) in dry dimethylformamide (7 cm^3) was added slowly to a cooled (0 °C) suspension of NaH in dimethylformamide (8 cm^3) under nitrogen. 3-Bromo-2-methylpropene (1 cm^3, 9.9 mmol) was added dropwise from



a syringe and stirring continued at 0°C for 10 min and then at room temperature for a further 16 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (50 cm^3) at 0°C, then extracted with diethyl ether (4 times) and the combined ethereal phases washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to give a pale yellow liquid (1.02 g) which was then purified by column chromatography on silica gel using gradient mixtures of diethyl ether : hexane 1:9 to 1:4 as eluent to afford *N,N-Bis(2-methylprop-2-enyl)benzamide* **71a** (0.66 g, 64 %) as a pale yellow liquid; ν_{max} cm^{-1} (film) 1812, 1640, 1578, 1495, 1375, 1294, 1266, 1242, 1149; δ_H (270 MHz, $CDCl_3$) 1.57 (3H, s, CH_3), 1.78 (3H, s, CH_3), 3.72 (2H, s, CH_2N), 4.11 (2H, s, CH_2N), 4.80-4.97 (4H, m, $=CH_2$), 7.33-7.46 (5H, m, ArH); δ_C (67.5 MHz, $CDCl_3$) 20.0 (CH_3), 20.1 (CH_3), 49.1 (CH_2), 53.5 (CH_2), 112.2 (CH_2), 126.3 (CH), 128.3 (CH), 129.4 (CH), 136.3 (C), 140.1 (C), 140.4 (C), 172.0 (C); *m/z* (FAB) 230.1538 (M+H, for $C_{15}H_{20}NO$ required 230.1545).

5.3.2 Synthesis of Polymer-Supported Initiators

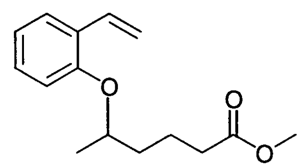
Methyl (±)-5-hydroxyhexanoate 56 [96]



Sodium methoxide (2.37 g, 44 mmol) was added at 0°C to a stirred solution of 5-hexanolactone **55** (4.8 cm^3, 44 mmol) in methanol (30.0 cm^3) and then stirred at room temperature for 20 h. The reaction mixture was then evaporated to remove methanol, shaken with diethyl ether and water, and the aqueous phase was extracted with diethyl ether (4 times). The combined organics were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to give *methyl (±)-5-hydroxyhexanoate* **56** (4.01 g, 63%) as a colourless viscous liquid; ν_{max} cm^{-1}(film) 3420-3360, 1720, 1245, 1165; δ_H (400 MHz, $CDCl_3$) 1.16 (3H, d, *J* 6.2, CH_3CHOH), 1.38-

1.48 (2H, m, CH_2CHOH), 1.57-1.77 (2H, m, $CH_2CH_2CH_2$), 2.10 (1H, br s, OH), 2.32 (2H, t, *J* 7.3, CH_2CO), 3.64 (3H, s, CH_3O), 3.78 (1H, sextet, *J* 6.2, C*H*OH), δ_C (100 MHz, $CDCl_3$) 21.3 (CH_2), 23.7 (CH_3), 34.0 (CH_2), 38.7 (CH_2), 51.7 (CH_3), 67.5 (CH), 174.2 (C); *m/z* (FAB) 147 (M+H, 18 %), 129 (M+H-H_2O, 78), 115 (M+H-CH_3OH, 100), 97 (20).

Methyl (±)-5-(2-vinylphenoxy)hexanoate 59

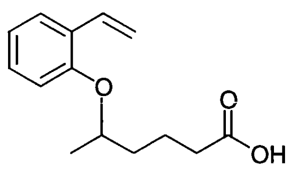


2-Hydroxycinnamic acid (2.00 g, 12 mmol) was decarboxylated at 210°C, under vacuum (1-2 mmHg) in Kugelrohr apparatus, previously lined with 1,4-benzoquinone (polymerisation inhibitor). 2-Vinylphenol **18** was collected in a flask externally cooled using dry ice/acetone. The resulting oil (1.46 g) containing 2-vinylphenol with 1,4-benzoquinone was used crude without further purification.

Diisopropyl azodicarboxylate (2.8 cm^3, 14 mmol) was added to a stirred solution of the above crude **18** (1.46 g), methyl 5-hydroxyhexanoate **56** (2.10 g, 14 mmol) and triphenylphosphine (3.78 g, 14 mmol) in tetrahydrofuran (20.0 cm^3) at 0°C. After 16 h, silica gel (15 g) was added and the reaction mixture was concentrated. Column chromatography on silica gel using 5:95 ethyl acetate : hexane as eluent afforded the *ether* **59** as a colourless oil (0.60 g, 20% based on 2-hydroxycinnamic acid); ν_{max} cm^{-1} (film) 1725, 1610, 1585, 1560, 1280, 1230; δ_H (400 MHz, $CDCl_3$) 1.32 (3H, d, *J* 6.0, CH_3CH), 1.60-1.90 (4H, m, C_2H_4CH), 2.37 (2H, t, *J* 7.0, CH_2CO), 3.67 (3H, s, CH_3O), 4.41 (1H, tq, *J* 6.0, 5.7, CHO), 5.24 (1H, dd, *J* 11.0, 1.5, CH=C$H_{cis}$$H_{trans}$), 5.73 (1H, dd, *J* 18.0, 1.5, CH=C$H_{cis}$$H_{trans}$), 6.87 (1H, d, *J* 8.0, ArH), 6.92 (1H, t, *J* 8.0, ArH), 7.07 (1H, dd, *J* 18.0, 11.0, C*H*=CH_2), 7.21 (1H, t, *J* 8.0, ArH), 7.50 (1H, d, *J* 8.0, ArH), δ_C (100 MHz, $CDCl_3$) 20.0 (CH_3), 21.3 (CH_2), 34.2 (CH_2), 36.2 (CH_2), 51.7 (CH_3), 74.1 (CH), 113.8 (CH), 114.0 (CH_2), 120.6 (CH), 126.6 (CH), 127.9 (C), 128.7 (CH), 131.9

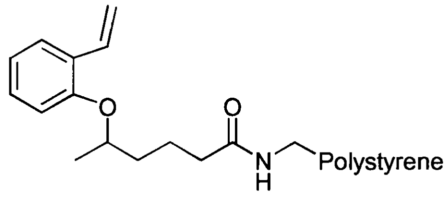
(CH), 155.1 (C), 173.9 (C); *m/z* (FAB): 249.1492 (M+H, $C_{15}H_{21}O_3$ requires 249.1491), 129 (100 %), 97 (13).

(±) 5-(2-vinylphenoxy)hexanoic acid 60



Aqueous sodium hydroxide (1 M, 4.5 cm^3, 4.5 mmol) was added to a stirring solution of methyl 5-(2-vinylphenoxy)hexanoate **59** (0.90 g, 3.6 mmol) in 1,4-dioxane (8.0 cm^3). After 24 h, the reaction mixture was concentrated and partitioned between diethyl ether and water. The aqueous phase was extracted twice with diethyl ether and the combined ethereal phases were washed with water (twice). The combined aqueous phases were then acidified and extracted with diethyl ether (4 times). The combined organics were washed with brine, dried over Na_2SO_4 and concentrated to give the *acid* **60** (0.65 g, 77%) as a viscous, pale yellow oil; ν_{max} cm^{-1} (film) 3060-2860, 1695, 1610, 1585, 1565, 1230, 1100; δ_H (400 MHz, $CDCl_3$) 1.31 (3H, d, *J* 6.2, CH_3), 1.65-1.90 (4H, m, C_2H_4CHO), 2.41 (2H, t, *J* 7.0, CH_2CO), 4.41 (1H, tq, *J* 6.2, 5.8, OCH), 5.23 (1H, dd, *J* 11.1, 1.6, $CHCH_{cis}H_{trans}$), 5.72 (1H, dd, *J* 18.0, 1.6, $CHCH_{cis}H_{trans}$), 6.86 (1H, d, *J* 7.4, ArH), 6.91 (1H, t, *J* 7.4, ArH), 7.06 (1H, dd, *J* 18.0, 11.1, $CH{=}CH_2$), 7.20 (1H, td, *J* 7.4, 1.6, ArH), 7.49 (1H, dd, *J* 7.4, 1.6, ArH), δ_C (100MHz, $CDCl_3$) 19.8 (CH_3), 20.9 (CH_2), 33.9 (CH_2), 35.9 (CH_2), 73.9 (CH), 113.7 (CH), 113.9 (CH_2), 120.5 (CH), 126.4 (CH), 127.7 (C), 128.6 (CH), 131.7 (CH), 154.9 (C), 179.2 (C); *m/z* (FAB) 257 (M+Na, 25%), 235.1330 (M+H, $C_{14}H_{19}O_3$ requires 235.1334), 234 (99), 97 (31).

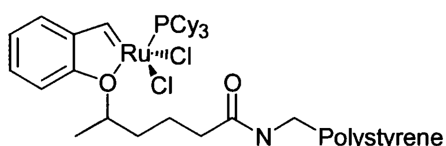
Polystyrene-supported ligand 61-P



Diisopropylcarbodiimide (0.25 cm^3, 1.62 mmol) was added to a stirring solution of (±) 5-(2-vinylphenoxy)hexanoic acid **60** (380 mg, 1.62

mmol) and 1-hydroxybenzotriazole (220 mg, 1.62 mmol) in dimethylformamide : dichloromethane 1:1 (4.00 cm^3). The reaction was stirred for ten minutes at room temperature, then added to a suspension of amino-methyl polystyrene resin (Advanced ChemTech, 250 mg, approximately 1.3 mmol/g, 0.32 mmol) in dimethylformamide : dichloromethane 1:1 (3.00 cm^3). The vessel was then sealed and the mixture agitated by vertical rotation on a blood tube rotator for 12 hours at room temperature, affording a resin that was negative to Kaiser analysis. The resin was then washed with 3 x 5.00 cm^3 portions of dichloromethane, tetrahydrofuran, dimethylformamide, dimethylformamide : methanol 1:1, dimethylformamide, tetrahydrofuran, dichloromethane successively and the resulting resin then dried under vacuum to give a polystyrene-supported ligand **61-P**. TentaGel- and Argopore-supported ligands **61-T** and **61-A** were prepared in the same way, starting from amino-TentaGel and amino-Argopore supports (respectively).

General procedure for polymer-supported initiators 62-P, 62-T, 62-A and 78-P

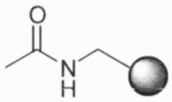


A solution of Grubb's catalyst **18** (25 mg, 0.03 mmol) in degassed 1,2-dichloroethane (1 cm^3) was added to a suspension of the above polystyrene-supported ligand **61-P** (0.32 mmol) in the same solvent (1 cm^3). The vessel was sealed and the mixture agitated by vertical rotation for 2 hours at room temperature and the resin then washed with dichloromethane until the filtrate was clear and dried under vacuum. The procedure was repeated four more times to afford *polystyrene-supported initiator* **62-P** as brown beads; phosphorus analysis revealed the loading of **62-P** to be 0.20 mmol/g (the batch of resin obtained by loading equimolar amount of Grubbs' catalyst afforded a resin of lower loading 0.12 mmol/g).

The same procedure was used to prepare **TentaGel-supported initiator 62-T** (loading with equimolar quantity of Grubbs' catalyst **18**) and **Argopore-supported initiator 62-**

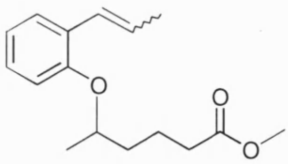
A (five times 10 mol% **18**) from corresponding polymer-supported ligands **61-T** and **61-A**. To prepare **polystyrene-supported initiator 78-P**, polymer-supported ligand **77-P** (page 136) was loaded with five 10 mol% portions of Grubbs' catalyst **18**.

N-acetyl amidomethyl polystyrene



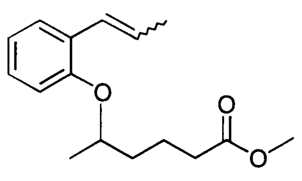
Dimethylformamide (1 cm^3) was added to aminomethyl polystyrene (100 mg, 0.13 mmol) in a plastic tube fitted with a frit. A solution of acetic anhydride (133 mg, 1.30 mmol) in dimethylformamide (2 cm^3) was added to the swollen resin, sealed and agitated by vertical rotation for 2 hours. As Kaiser test for amines was still positive, the reaction was continued for the total time of 24 h, when the Kaiser analysis was negative. The resin was filtered and washed multiple times with dichloromethane (100 cm^3) and dried in vacuum. Dichloromethane (1 cm^3) was then added to the resin followed by a solution of Grubbs' catalyst **18** (107 mg, 0.13 mmol) in dichloromethane (2 cm^3), the tube sealed and agitated by vertical rotation for 2h. The liquid was then filtered off, resin washed several time with dichloromethane, dried and then tested for ring-closing metathesis of benzyl *N,N*-diallylcarbamate **69** following the standard procedure for testing the resin.

(Z/E)-Methyl (±)-5-(2-(prop-1-enyl)phenoxy)hexanoate 75



Diisopropyl azodicarboxylate (9.6 cm^3, 49 mmol) was added to a stirring solution of 2-(prop-1-enyl)phenol (6.60 g, 49 mmol), methyl 5-hydroxyhexanoate (6.00 g, 41 mmol) and triphenylphosphine (12.90 g, 49 mmol) in tetrahydrofuran (50 cm^3) at 0°C under nitrogen. The reaction mixture was warmed to room temperature and stirring continued for 24 h. Silica gel was added, the reaction mixture concentrated and the resulting pad subjected to column chromatography on silica gel using 5 : 95 ethyl acetate : hexane as eluent afforded the *ether* **75** as a colourless oil (5.76 g, 53 %); approximately *cis* / *trans* 25 : 75 % (^{1}H NMR integration); ν_{max} cm^{-1} (film) 1739, 1596,1485, 1375, 1240, 1170, 1135; *cis* isomer δ_H (400 MHz, CDCl$_3$) 1.30 (3H, d, *J* 5.9, C*H*$_3$CHO), 1.60-1.87 (7H, m, C$_2$*H*$_4$CH and C*H*$_3$CH=CH), 2.32-2.40 (2H, m, CH$_2$CO), 3.65 (3H, s, CH$_3$O), 4.37 (1H, sextet, *J* 5.9, CHO), 5.79 (1H, dq, *J* 11.7, 7.0, CH=C*H*CH$_3$), 6.54 (1H, dd, *J* 11.7, 1.6, CH=C*H*Ph), 6.81-6.94 (2H, m, ArH), 7.18 (1H, td, *J* 8.1, 1.9 ArH), 7.27 (1H, dd, *J* 8.1, 1.9 ArH), *trans* isomer δ_H (400 MHz, CDCl$_3$) 1.31 (3H, d, *J* 5.9, C*H*$_3$CHO), 1.73 (4H, m, C$_2$*H*$_4$CH), 2.32-2.40 (2H, m, C*H*$_2$CO), 3.66 (3H, s, C*H*$_3$O), 4.37 (1H, sextet, *J* 5.9, CHO), 6.20 (1H, dq, *J* 16.0, 6.6, CH=C*H*CH$_3$), 6.71 (1H, dd, *J* 16.0, 1.6, CH=C*H*Ph), 6.81-6.94 (2H, m, ArH), 7.13 (1H, td, *J* 8.0, 1.6, ArH), 7.40 (1H, dd, *J* 8.0, 1.6 ArH); δ_C (100 MHz, CDCl$_3$) 19.0 (CH$_3$), 19.8 (CH$_3$), 21.1 (CH$_2$), 34.0 (CH$_2$), 35.9 (CH$_2$), 51.5 (CH$_3$), 73.9 (CH), 113.5 (CH), 120.5 (CH), 125.7 (CH), 126.1 (C), 126.2 (CH), 126.3 (CH), 127.4 (CH), 130.1 (CH), 154.3 (C), 173.7 (C); *m/z* (FAB) 263.1640 (M+H, for $C_{16}H_{23}O_3$ required 263.1647), 262 (40 %), 129 (100).

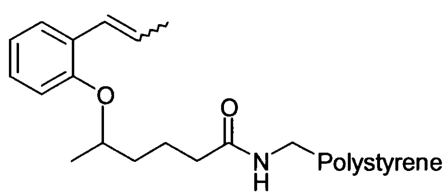
(±)-5-(2-(Prop-1-enyl)phenoxy)hexanoic acid 76



Aqueous sodium hydroxide (1 M, 16.5 cm^3, 16.5 mmol) was added to a stirring solution of methyl (±)-5-(2-(Prop-1-enyl)phenoxy)hexanoate **75** (3.60 g, 13.7 mmol) in 1,4-dioxane (20.0 cm^3). After 20 h, the reaction mixture was concentrated and partitioned between diethyl ether (50 cm^3) and water (50 cm^3). The aqueous phase was washed twice with diethyl ether and the combined ethereal phases extracted with water (twice). The combined aqueous phases were then acidified (hydrochloric acid, 1M) and extracted with diethyl ether (4 times). The combined organics were washed with brine, dried over Na_2SO_4 and concentrated to give the *acid* **76** (2.91 g, 86 %) as a viscous, pale yellow oil; approximately *cis* / *trans* isomer 25 : 75 % (^{1}H NMR integration); ν_{max} cm^{-1} (film)

3035-2930, 1709, 1596, 1239, 1100; *cis* isomer δ_H (400 MHz, $CDCl_3$) 1.30 (3H, d, *J*, 5.9, CH_3CHO), 1.65-1.86 (7H, m, C_2H_4CHO and CH_3CH=CH), 2.36-2.45 (2H, m, CH_2CO), 4.38 (1H, sextet, *J* 5.9, OCH), 5.80 (1H, dq, *J* 11.7, 7.0, CH=C*H*CH_3), 6.55 (1H, dd, *J* 11.7, 1.6 CH=C*H*Ph), 6.81-6.95 (2H, m, ArH), 7.18 (1H, td, *J* 8.1, 1.9, ArH), 7.28 (1H, dd, *J* 8.1, 1.9, ArH), *trans* isomer δ_H (400 MHz, $CDCl_3$) 1.31 (3H, d, *J*, 5.9, CH_3CHO), 1.65-1.86 (4H, m, C_2H_4CHO), 1.90 (3H, dd, *J* 6.6, 1.6, CH_3CH=CH), 2.36-2.45 (2H, m, CH_2CO), 4.38 (1H, sextet, *J* 5.9, OCH), 6.21 (1H, dq, *J* 16.0, 6.6, CH=C*H*CH_3), 6.72 (1H, dd, *J* 16.0, 1.6 CH=C*H*Ph), 6.81-6.95 (2H, m, ArH), 7.14 (1H, td, *J* 8.0, 1.6, ArH), 7.41 (1H, dd, *J* 8.0, 1.6, ArH); δ_C (100MHz, $CDCl_3$) 19.4 (CH_3), 20.2 (CH_3), 21.2 (CH_2), 34.2 (CH_2), 36.2 (CH_2), 74.3 (CH), 114.0 (CH), 120.9 (CH), 125.9 (CH), 126.2 (CH), 126.6 (CH), 127.8 (CH), 128.4 (C), 154.6 (C), 179.5 (C); *m/z* (FAB) 249.1478 (M+H, for $C_{15}H_{21}O_3$ required 249.1491), 248 (64 %), 134 (100), 115 (64).

(±) 5-[2-(2-propenyl)-phenoxy]-hexanoic acid amido polystyrene 77-P



Diisopropylcarbodiimide (1.00 cm^3, 6.5 mmol) was added to a stirring solution of (±) 5-[2-(2-propenyl)-phenoxy] hexanoic acid **76** (1.61 g, 6.5 mmol) and 1-hydroxybenzotriazole (0.88 g, 6.5 mmol) in dimethylformamide : dichloromethane 1:1 (4 cm^3). The reaction was stirred for ten minutes at room temperature, then added to a suspension of amino-methyl polystyrene resin (Advanced ChemTech, 1.00 g, approximately 1.3 mmol/g, 1.3 mmol) in dimethylformamide : dichloromethane 1:1 (3 cm^3). The vessel was then sealed and the mixture agitated by vertical rotation on a blood tube rotator for 12 hours at room temperature, affording a resin that was negative to Kaiser analysis. The resin was then washed with 3 x 5 cm^3 portions of dichloromethane, tetrahydrofuran, dimethylformamide, dimethylformamide : methanol 1:1, dimethylformamide, tetrahydrofuran, dichloromethane successively. The resulting resin was then dried under vacuum.

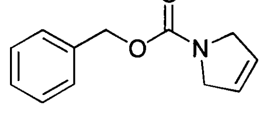
5.3.3 Testing of the Resins

General procedure for ring-closing metathesis using supported initiators

All reactions were performed in 3 cm^3 plastic solid phase synthesis tubes fitted with a glass frit using general laboratory grade dichloromethane, methanol, toluene or D_2O without further purification.

Diene (0.1 or 0.2 mmol) in dichloromethane (0.5 cm^3) was added to supported initiator (25 mg). The tube was then sealed and agitated by 360° rotation on a blood tube rotator at room temperature or immersed in a water bath at 40 °C without agitation (in the case of second generation initiators) for 90 minutes unless otherwise stated. The supernatant was then collected by filtration and the resin remaining in the tube washed with dichloromethane (3 x 2 cm^3). The organics were then combined and concentrated, then analysed by ^1H NMR. Yields were initially estimated by comparison of the integration for CH_2 peaks of starting material *versus* product.

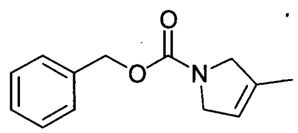
Ring-closing olefin metathesis of benzyl *N,N*-diallylcarbamate 69a



Benzyl *N,N*-diallylcarbamate **69a** (142 mg, 0.612 mmol) in dichloromethane (3 cm^3) was added to the polystyrene-supported initiator **62-P** (153 mg, 0.031 mmol, 5 mol%) in a plastic tube with a frit, sealed and agitated by vertical rotation for 4 hours at room temperature. The supernatant was then collected by filtration, the resin washed with dichloromethane and the combined organics concentrated and purified by column chromatography using gradient mixtures of diethyl ether : hexane (1:19 to 1:4) to isolate *N-benzyloxycarbonyl-2,5-*

dihydropyrrole **69b** (130 mg, 91 %) as colourless oil; ν_{max} cm^{-1} (film) 1707, 1624, 1587, 1538, 1363, 1211, 1116, 1003; δ_H[123] (270 MHz, $CDCl_3$) 4.14-4.24 (4H, m, CH_2NCH_2), 5.16 (2H, s, CH_2O), 5.72-5.84 (2H, m, CH=CH), 7.26-7.41 (5H, m, ArH); δ_C (100 MHz, $CDCl_3$) 53.3 (CH_2), 53.8 (CH_2), 67.1 (CH_2), 125.8 (CH), 126.0 (CH), 128.0 (CH), 128.1 (CH), 128.7 (CH), 137.1 (C), 154.7 (C); *m/z* (FAB) 204 (M+H, 65 %), 112 (M-C_7H_7, 18), 91 ($C_7H_7^+$, 100).

1-Benzyloxycarbonyl-3-methyl-2,5-dihydropyrrole 71b

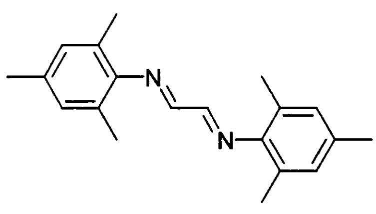


A solution of benzyl *N*-allyl-*N*-(2-methylallyl)carbamate **71a** (54 mg, 0.22 mmol) in reagent grade dichloromethane (1 cm^3) was added to the resin **90-P** (25 mg, unknown loading) in a plastic tube with a frit and submersed in a water bath at 40 °C and heated for 5 h. The reaction mixture was then filtered, the resin washed with dichloromethane, the washings combined with the filtrate, concentrated and the residue analysed by NMR (81 % conversion). The NMR sample was reconcentrated and the cyclised product of ring-closing metathesis isolated by column chromatography, eluting with gradient mixtures of diethyl ether : hexane 1:19 to 1:9, to give *1-Benzyloxycarbonyl-3-methyl-2,5-dihydropyrrole* **71b** (26 mg, 54 %) as an oil; ν_{max} cm^{-1}(film) 1708, 1664, 1586, 1362, 1329, 1237, 1212, 1164; δ_H (400 MHz, $CDCl_3$) 1.74 (1.5 H, s, CH_3), 1.76 (1.5 H, s, CH_3), 4.03-4.11 (2H, m, CH_2N), 4.12-4.20 (2H, m, CH_2N), 5.15 (2H, s, OCH_2Ph), 5.33-5.37 (0.5 H, m, CH=C), 5.38-5.42 (0.5 H, m, CH=C), 7.27-7.40 (5H, m, ArH); δ_C (100 MHz, $CDCl_3$) 14.7 (CH_3), 14.8 (CH_3), 53.7 (CH_2), 54.1 (CH_2), 56.5 (CH_2), 56.9 (CH_2), 67.0 (CH_2), 67.1 (CH_2), 119.5 (CH), 119.6 (CH), 128.0 (CH), 128.1 (CH), 128.6 (CH), 135.3 (C), 135.5 (C), 137.2 (C), 154.7 (C), (additional peaks in ^1H and ^{13}C spectra due to restricted rotation of C-N bond, two rotamers observed); *m/z* (FAB) 218.1174 (M+H, for $C_{13}H_{16}NO_2$ required 218.1181), 91 (C_7 H_7^+, 100 %).

5.4 Phosphine-Free Polymer-Supported Alkylidene Ruthenium For Olefin Metathesis – the Second Generation

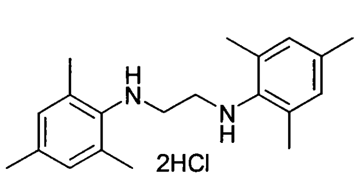
5.4.1 Synthesis of Second Generation Polymer-Supported Initiators

Glyoxal *N,N'*-bis(2,4,6-trimethylphenyl)imine 85[101]



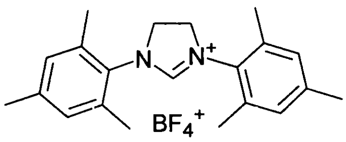
Glyoxal (40 % solution in water, 18.15g, 0.125 mol) was added to a solution of 2,4,6-trimethylaniline in 1-propanol (150 cm^3) followed by a 50 cm^3 of 1-propanol and 25 cm^3 water and the mixture stirred at 60°C for one hour. After approximately 15 minutes yellow precipitate started to form. The reaction mixture was then allowed to cool to room temperature and stirred for further 18 h. Water (100 cm^3) was then added, the yellow precipitate collected by filtration, washed with water and dried in vacuum at 50°C. The remaining filtrate was evaporated and the residue recrystallised from ethyl acetate and to give *N,N'-dimesity glyoxalimine* **85** as long yellow needles (32.93 g, 90 %); mp 158°C (lit.[101] mp 157-158°C); ν_{max} cm^{-1} (KBr) 3000-2800, 1617br, 1477br, 1374, 1202, 1140, 1032; δ_H (400 MHz, $CDCl_3$) 2.17 (12H, s, 2,6-CH_3), 2.30 (6H, s, 4-CH_3), 6.92 (4H, s, ArH), 8.11 (2H, s, CH=N); δ_C (100 MHz, $CDCl_3$) 18.3 (CH_3), 20.9 (CH_3), 126.4 (C), 128.8 (CH), 134.1 (C), 147.3 (C), 163.3 (CH); *m/z* (FAB) 293 (81%), 277 (100), 146 (35).

***N,N'*-Bis(2,4,6-trimethylphenyl)ethane-1,2-diamine dihydrochloride 86**[101]



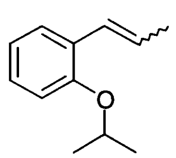
Sodium borohydride (7.57 g, 0.20 mol) was added in portions to a suspension of *N,N'*-dimesitylglyoxal imine **85** (15.00 g, 0.05 mol) in methanol (150 cm^3) at 0°C and then stirred at room temperature for 3h. The reaction was quenched with ice/water (100 cm^3) and 3M aqueous hydrochloric acid added until pH ~1, resulting in a white precipitate that was collected by filtration. The precipitate was then washed with water and dried in vacuum at 50°C to give *N,N'-dimesitylethylenediamine dihydrochloride* **86** (15.82 g, 84 %) as a white powder; mp 210°C (lit.[101] mp 250°C); ν_{max} cm^{-1} (KBr) 3428br, 3256, 2663, 2464, 1668, 1611, 1220, 1137; δ_H (400 MHz, $(CD_3)_2SO$) 2.17 (6H, s, 4-CH_3), 2.30 (12H, s, 2,6-CH_3), 3.25 (4H, s, CH_2), 6.83 (4H, s, ArH); δ_C (100 MHz, $(CD_3)_2SO$) 18.2 (CH_3), 20.2 (CH_3), 47.5 (CH_2), 129.4 (CH), 130.3 (C), 133.1 (C), 139.0 (C); *m/z* (FAB) 297 (M+H, 100 %), 148 (46).

1,3-Bis(2,4,6-trimethylphenyl)-4,5-dihydro-3H-imidazol-1-ium tetrafluoroborate 87 [101,102]



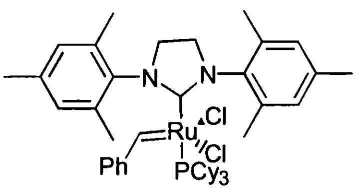
N,N'-Dimesitylethylenediamine dihydrochloride **86** (10.00 g, 27 mmol) was shaken with saturated aqueous Na_2CO_3 (30 cm^3) and chloroform (30 cm^3) and the layers separated. The aqueous phase was then extracted twice with chloroform and the combined organics washed with brine, dried over anhydrous Na_2SO_4 and evaporated to give the free base of diamine **86**. Triethyl orthoformate (4.5 cm^3, 27 mmol), ammonium tetrafluoroborate (2.84 g, 27 mmol) and diamine **86** (8.41 g, 27 mmol) were then stirred at 130°C for 3h. The reaction mixture was evaporated and the residue recrystallised from ethanol to give *1,3-dimesitylimidazolinium tetrafluoroborate* **87** (6.18 g, 58 %) as white needle-like crystals; mp 288-290 °C (lit.[101] mp >250 °C); ν_{max} cm^{-1} (KBr) 3094, 1629, 1394, 1313, 1269, 1214, 1058br; δ_H[101](400 MHz, $(CD_3)_2SO$) 2.27 (6H, s, 4-CH_3), 2.33 (12H, s, 2,6-CH_3), 4.42 (4H, s, CH_2), 7.07 (4H, s, ArH), 8.96 (1H, s, NCH=N); δ_C[101] (100 MHz, $(CD_3)_2SO$) 18.0 (CH_3), 21.4 (CH_3), 51.7 (CH_2), 130.1 (CH), 131.5 (C), 136.0 (C), 140.3 (C), 160.8 (CH); *m/z* (FAB) 307 (M^+, 100 %).

1-(1-Methylethoxy)- 2-(prop-1-enyl)benzene 88



Freshly ground potassium carbonate (18.00g, 130 mmol) was added at 0°C to a solution of 2-(2-propenyl)-phenol **74** (4.0 cm^3, 31 mmol) in acetone (50 cm^3) with a few drops of water and stirred to form a fine suspension. 2-Bromopropane (3.6 cm^3, 38 mmol) was then added and the reaction mixture stirred at reflux temperature overnight. As the reaction did not go to completion 18-crown-6 (a few mg) and more 2-bromopropane (3.0 cm^3, 32 mmol) were added and stirring continued for further 100 hours. The mixture was then concentrated and the residue shaken with water and extracted into dichloromethane (three times) and concentrated in vacuum to leave orange-yellow oil, which was then distilled using Kugelrohr apparatus (75 °C, 2 mmHg) to give *1-isopropoxy-2-(prop-1-enyl)benzene* **88** (4.62 g, 84 %) as a colourless oil; *cis / trans* isomer 25 : 75 % approximately (^{1}H NMR integration); ν_{max} cm^{-1} (film) 3032, 1654, 1596, 1239; *cis* isomer δ_H(400 MHz, $CDCl_3$) 1.33 (6H, d, *J* 6.1, 2xCH_3), 1.84 (3H, dd, *J* 7.0, 2.0, C*H*$_3$CH=CH), 4.52 (1H, septet, *J* 6.1, CHO), 5.79 (1H, dq, *J* 11.4, 7.0, CH=C*H*CH_3), 6.55 (1H, dq, *J* 11.4, 2.0, CH=C*H*Ph), 6.83-6.94 (2H, m, ArH), 7.18 (1H, td, *J* 7.6, 1.6, ArH), 7.28 (1H, dd, *J* 7.6, 1.6, ArH), *trans* isomer δ_H(400 MHz, $CDCl_3$) 1.35 (6H, d, *J* 6.1, 2xCH_3), 1.89 (1H, dd, *J* 6.7, 2.0, C*H*$_3$CH=CH), 4.52 (1H, septet, *J* 6.1, CHO), 6.22 (1H, dq, *J* 16.0, 6.7, CH=C*H*CH_3), 6.72 (1H, dq, *J* 16.0, 2.0, CH=C*H*Ph), 6.83-6.94 (2H, m, ArH), 7.13 (1H, td, *J* 8.0, 1.7, ArH), 7.40 (1H, dd, *J* 8.0, 1.7, ArH); δ_C (100MHz, $CDCl_3$) 19.1 (CH_3), 22.3 (2xCH_3), 70.8 (CH), 114.1 (CH), 120.5 (CH), 125.7 (CH), 126.3 (CH), 127.4 (CH), 127.6 (CH), 130.1 (C), 154.4 (C); *m/z* (EI) 176.1204 (M^+, for $C_{12}H_{16}O$ required 176.1201), 134 (100 %), 133 (48), 119 (44), 91 ($C_7H_7^+$, 26), 77 ($C_6H_5^+$, 18).

Benzylidene [1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine] ruthenium(IV) dichloride 20 [67,103]

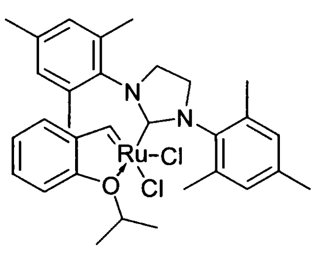


Method 1: [67] The reagents were dried overnight over P_2O_5 under vacuum and the solvents dried and purged with argon prior to use. 1,3-Bis(2,4,6-trimethylphenyl) imidazolinium tetrafluoroborate **87** (294 mg, 0.75 mmol) was suspended in dry tetrahydrofuran (5 cm^3) in a 10 cm^3 round-bottomed flask under argon. This mixture was then treated with a solution of potassium *tert*-butoxide (84 mg, 0.75 mmol) in tetrahydrofuran (5 cm^3) *via* cannula at room temperature. This mixture was then *immediately* transferred by cannula (additional 2 cm^3 tetrahydrofuran used to rinse) to a second vessel containing a solution of Grubbs' catalyst **18** (500 mg, 0.61 mmol) in benzene (10 cm^3, previously purged with argon). The resulting mixture was refluxed at 80 °C for 30 min and then cooled to room temperature. All manipulations from this point on, were carried out in air with reagent grade solvents. The solvents were removed at reduced pressure, leaving a red-brown solid residue that was then loaded onto a column of silica gel and eluted with 1:8 diethyl ether : hexane. Concentration of the product fractions *in vacuo* afforded a *second generation ruthenium catalyst* **20** (150 mg, 29 %) as a pink-brown solid.

Method 2: [103] All reagents were dried and purged with argon prior to use. A 50 cm^3 V-bottomed two neck flask equipped with a reflux condenser was charged under argon with a 25 % w/w solution of potassium *tert*-pentoxide in toluene (1.1 cm^3, 1.8 mmol) and the solution was then stirred vigorously under vacuum to remove toluene. The flask was purged with argon and 1,3-bis(2,4,6-trimethylphenyl)imidazolinium tetrafluoroborate **87** (710 mg, 1.8 mmol) and hexane (20 cm^3) were added. The resulting white suspension was stirred under argon at room temperature for 90 min during which time the suspension slowly turned into a slightly turbid solution. Grubbs' catalyst **18**

(1.00 g, 1.2 mmol) was then added as a solid and the mixture stirred at 60 °C for 2 h. The colour of the reaction mixture changed from dark purple to pink-brown. The reaction was then cooled to room temperature, filtered, the precipitate washed with hexane and then methanol, and then dried to give *second generation ruthenium catalyst* **20** (0.95 g, 93 %) as a fine pink-brown powder; mp 173–175 °C (no lit. mp data); ν_{max} cm^{-1}(KBr) 2927br, 2848, 1609, 1483, 1264, 1244, 1034br; δ_H[57](270 MHz, $CDCl_3$) 0.86 – 2.75 (51 H, m, CyH and 6xCH_3), 3.98 (4H, s, CH_2NCH_2), 7.02 – 7.39 (9H, m, ArH), 19.13 (1H, s, CH=Ru); δ_P[57] (109.3 MHz, $CDCl_3$) 29.66; *m/z* (FAB) 850.2 (M+H, 26 %), 848.2 (32), 370.2 (100), 281.2 (74).

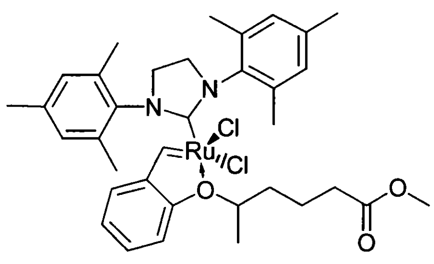
[2-(Prop-2-yloxy)benzylidene][1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine]ruthenium(IV) dichloride 22[67,124]



Method 1 (modified method of Morgan *et al.*): [124] A solution of potassium *tert*-butoxide (28 mg, 0.25 mmol) in tetrahydrofuran (2 cm^3) was added, under nitrogen, via cannula to the suspension of 1,3-bis-(2,4,6-trimethylphenyl)-4,5-dihydro-3H-imidazol-1-ium tetrafluoroborate **87** (100 mg, 0.25 mmol) in tetrahydrofuran (5 cm^3). This mixture was then immediately transferred by cannula into a third flask containing a solution of Grubbs' catalyst **18** (150 mg, 0.18 mmol) in dry toluene (5 cm^3) and the resulting mixture heated to 80 °C for 30 min under nitrogen. The mixture was then cooled and 2-(2-propenyl)-1-isopropyloxy benzene (44 mg, 0.25 mmol) added and the reaction stirred at 45 °C for a further 14 h, followed by concentration *in vacuo* to give a brown residue. Purification by silica gel chromatography using 9:1 hexane : diethyl ether as eluent afforded initiator **22** (36 mg, 32 %) as a green solid.

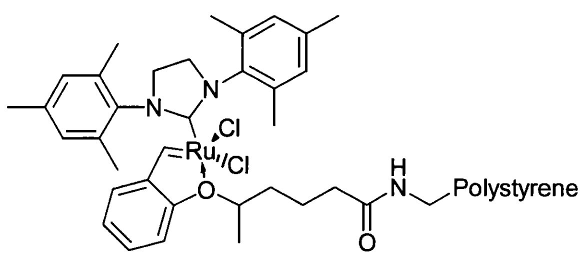
Method 2 (by Garber *et al.*):[67] Second generation catalyst **20** (60 mg, 0.07 mmol) and CuCl (7 mg, 0.07 mmol) were dissolved in degassed dichloromethane (2 cm^3). A solution of 2'-isopropoxystyrene **88** (12 mg, 0.07 mmol) in dichloromethane (1 cm^3) was added *via* syringe to the resulting deep red solution at room temperature and under argon. The flask was then equipped with a condenser and stirred at 40 °C for 1 h. From this point onwards all manipulations were done in air with reagent grade solvents. The reaction mixture was filtered through a plug of glass wool to remove insoluble copper-phosphine precipitates and then concentrated *in vacuo* to a dark-brown solid residue. This residue was then dissolved in a small amount of 1:1 hexane : dichloromethane and loaded onto a wide silica gel column and eluted with the same solvent mixture as a bright green band from the coulumn. All collected fractions were combined and concentrated to give *[2-(Prop-2-yloxy)benzylidene][1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine]ruthenium(IV) dichloride* **22** (39 mg, 89 %) as a green solid; δ_H[67] (400 MHz, $CDCl_3$) 1.27 (6H, d, *J* 6.1, $(CH_3)_2CHO$), 2.40 (6H, s, Mes 4-CH_3), 2.48 (12H, s, Mes 2,6-CH_3), 4.17 (4H, s, $N(CH_2)_2N$), 4.89 (1H, septet, *J* 6.1, $(CH_3)_2CHOPh$), 6.68-6.88 (3H, m, ArH), 7.07 (4H, s, Mes ArH), 7.47 (1H, t, *J* 8.6, ArH), 16.42 (1H, s, Ru=CHPh).

[2-(5-Methoxy-1-methyl-5-oxopentoxy)benzylidene][1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine]ruthenium(IV) dichloride 89



Modified method by Garber *et al.*:[67] Second generation catalyst **20** (102 mg, 0.12 mmol) and methyl 5-(2-(prop-1-enyl)phenoxy)hexanoate **75** (32 mg, 0.12 mmol) were dissolved in degassed dichloroethane (4 cm^3) and stirred under argon, first at 50 °C for 5 h and then at room temperature for another 10 h. From this point all manipulations were done in air with reagent grade solvents. The reaction mixture was then concentrated to a dark-brown solid residue, which was dissolved in a small amount of dichloromethane, absorbed onto approximately 0.5 cm^3 of silica gel and the solvent removed *in vacuo*. The dried silica gel was loaded onto a short silica gel column (approximately 15 cm^3 of silica) and the product eluted with diethyl ether : hexane 1:5 as a bright green band from the column. The combined fractions were concentrated affording *[2-(5-methoxy-1-methyl-5-oxopentoxy)benzylidene][1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine]ruthenium(IV) dichloride* **89** (51 mg, 60 %) as a green microcrystalline powder; mp 220-222 °C; ν_{max} cm^{-1} (KBr) 3445br, 1733, 1607, 1590, 1261, 1218, 1186, 1158; δ_H (270 MHz, $CDCl_3$) 1.25 (3H, d, *J* 6.1, C*H*$_3$CH-O), 1.42-1.61 (2H, m, CH_2), 1.82-2.00 (2H, m, CH_2), 2.20 (2H, t, *J* 7.4, C*H*$_2$CO), 2.40 (6H, s, Mes 4-CH_3), 2.45 (12H, s, Mes 2,6-CH_3), 3.65 (3H, s, CH_3O), 4.13 (4H, s, $N(CH_2)_2N$), 4.60-4.74 (1H, m, CH-O), 6.74 (1H, d, *J* 8.2, ArH), 6.79-6.93 (2H, m, ArH), 7.06 (4H, s, Mes ArH), 7.47 (1H, td, *J* 8.2, 1.9, ArH), 16.53 (1H, s, CHRu); δ_C (68 MHz, $CDCl_3$) 18.4 (CH_3), 19.3(CH_3), 21.1 (CH_3), 21.3 (CH_2), 33.7 (CH_2), 34.1 (CH_2), 51.5 (CH_3), 51.7 (CH_2), 78.1 (CH), 112.9 (CH), 122.4 (CH), 122.8 (CH), 129.4 (CH), 129.6 (CH), 138.8 (C), 145.2 (C), 147.0 (C), 151.9 (C), 157.3 (C), 173.7 (C), 211.0 (C), 296.6 (CH); *m/z* (FAB) 713.1791 (M+H, for $C_{35}H_{45}N_2O_3Cl_2{}^{102}Ru$ required 713.1851), 712.1794 (M+H, for $C_{35}H_{45}N_2O_3Cl_2{}^{101}Ru$ required 712.1863), 677 (54 %), 548 (48), 405 (100).

Second-generation polystyrene-supported initiator 90-P

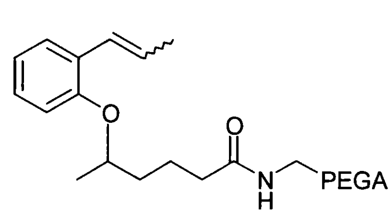


Method 1: A solution of benzylidene [1,3 - bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine] ruthenium (IV) dichloride **20** (55 mg, 0.07 mmol) in degassed 1,2-dichloroethane (2 cm^3) was added to a suspension of 5-(2-(prop-1-

enyl)phenoxy)hexanoic acid amidopolystyrene **77-P** (500 mg, 0.65 mmol) in dichloroethane (2 cm^3). The vessel was fitted with an improvised air condenser and the mixture immersed in a 40°C water bath and heated under nitrogen without stirring for 4 hours. The resin was then filtered through a frit and repeatedly washed with dichloromethane (approximately 50 cm^3) until the filtrate was clear, then dried under vacuum. The whole procedure was then repeated four more times to afford *second generation polystyrene-supported ruthenium initiator* **90-P** (550 mg) as a deep green resin.

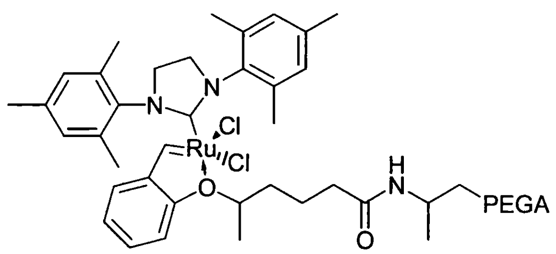
Method 2 with CuCl: A solution of Benzylidene[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidine]ruthenium(IV) dichloride **20** (326 mg, 0.38 mmol) in degassed 1,2-dichloroethane (2 cm^3) and solid copper(I) chloride (38 mg, 0.38 mmol) were added to a suspension of 5-(2-(prop-1-enyl)phenoxy)hexanoic acid amido polystyrene **77-P** (250 mg, no more than 0.33 mmol) in dichloroethane (1 cm^3). The vessel was fitted with an improvised air condenser and the mixture was immersed in a 40 °C water bath and heated under nitrogen flow without stirring for 2 hours. The reaction mixture was then removed form the water bath, sealed and agitated by rotation on a blood tube rotator for another 12 h. The resin was then filtered and repeatedly washed with dichloromethane (approximately 50 cm^3) until the filtrate was clear and dried under vacuum to afford *second generation polystyrene-supported ruthenium initiator* **90-P** (352 mg) as very dark green/black beads.

(±) 5-[2-(2-propenyl)phenoxy]hexanoic acid amido PEGA 77-PEGA



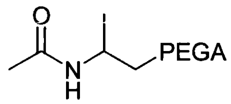
Method of Connon *et al.*:[98]: Amino-PEGA resin (Novabiochem 0.4 mmol/g of dry resin) preswollen in methanol (4.15 g) was weighed into a 15 cm^3 plastic tube with a frit and washed with successive 30 cm^3 portions of dimethylformamide, tetrahydrofuran and dichloromethane and dried in vacuum to give dry amino-PEGA resin (515 mg, 0.21 mmol) which was then left to swell in dimethylformamide (2.0 cm^3) for 1 h. In a separate flask, diisopropylcarbodiimide (160 μL, 1.03 mmol) was added to a solution of 1-hydroxybenzotriazole (139 mg, 1.03 mmol) and 5-(2-(prop-1-enyl)phenoxy)hexanoic acid **76** (256 mg, 1.03 mmol) in dimethylformamide (5.0 cm^3), stirred for ten minutes at room temperature and then added to a suspension of amino-PEGA resin in dimethylformamide (3.0 cm^3). The vessel was then sealed and agitated on a blood tube rotator for 16 hours at room temperature, followed by washing with dimethylformamide (7 x 5.0 cm^3). The solvent was then exchanged by repeated washing with dichloromethane. The solution of triethylamine (730 mg, 7.20 mmol) and *N,N*-dimethylaminopyridine (75 mg, 0.62 mmol) in dichloromethane (3.0 cm^3) was added, followed by acetic anhydride (0.6 cm^3, 6.20 mmol) in dichloromethane (1.0 cm^3) and the resulting mixture agitated for 15 h at room temperature on a blood tube rotator. The resin was then washed with consecutive 3 x 15 cm^3 portions of dichloromethane, dimethylformamide, water, dimethylformamide, dichloromethane and diethyl ether and dried under vacuum to afford *PEGA-supported ligand* **77-PEGA** (654 mg).

Second-generation PEGA-supported initiator 90-PEGA



Modified method of Connon *et al.*:[98] PEGA-supported ligand **77-PEGA** (654 mg, ~ 0.20 mmol) was suspended in dichloromethane (3 cm^3). To this was added second-generation Grubbs' catalyst **20** (18 mg, 0.02 mmol) in dichloromethane (2 cm^3), the resulting deep red mixture was heated at reflux for 4 h. The resin was then filtered, washed with several portions of dichloromethane, and dried. The procedure was then repeated a further four times, with a new portion of **20**. After five loadings the resin was washed with multiple volumes of dichloromethane until the filtrate ran clear, then three times with diethyl ether, then dried in vacuo to give *PEGA-supported second generation initiator* **90-PEGA** (715 mg) as a green resin.

Acetyl amido PEGA



Dried amino-PEGA resin (105 mg, 0.21 mmol) was preswollen in dichloromethane (1.0 cm^3) for 1h and a solution of triethylamine (94 mg, 0.92 mmol) and 4-dimethylaminopyridine (10 mg, 0.08 mg) in dichloromethane (1.0 cm^3) was added, followed by a solution of acetic anhydride (86 mg, 0.84 mmol) in dichloromethane (0.5 cm^3). The vessel was sealed and agitated by rotation on a blood tube rotator at room temperature for 16 h. The resin was then filtered and washed with 3 x 5 cm^3 of dichloromethane, dimethylformamide, water, dimethylformamide, dichloromethane and finally 3 x 5 cm^3 of diethyl ether, followed by drying *in vacuo*. This *N*-capped PEGA was then swollen in degassed 1,2-dichloroethane for 1h a solution of Grubbs' catalyst **18** (34 mg, 0.042 mmol) in 1,2-dichloroethane (2.0 cm^3) was added to it, the vessel was then sealed and agitated for 2 h at room temperature. The resin was filtered, washed with multiple portions of dichloromethane until filtrate ran clear and dried *in vacuo* to give *N-capped PEGA* as a pink resin.

6 REFERENCES

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